Attachment 12

Michigan State Police,
Biology Procedures and
Training Manuals

Biology Procedures & Training Manuals

Biology Procedures & Training Manuals

Biology Procedures Manual

Biology Training Manual

Biology Procedures Manual

1.0 Serology Procedures

1.1 Quality Control Quality Assurance

1.2 Saliva Detection

1.3 Blood Detection

1.4 Feces Detection

1.5 Semen and Seminal Fluid Detection

1.6 Urine Tests

1.7 Evidence Examination, Collection and Documentation

1.8 Digital Imaging

1.9 Hair Evaluations

1.10 Male DNA Screening of Sexual Assault Evidence

1.11 Body Fluid Identification Laboratory Reports

1.1 Quality Control Quality Assurance

1.1 Quality Control Quality Assurance

"DNA recovered from the following items was inconclusive for the possible presence of male DNA and was not processed further." LIST OF ITEMS.

1.11 Body Fluid Identification Laboratory Reports

The following table (provided as a link) is to be used for the generation of Body Fluid Identification laboratory reports. Each item examined should have its own results section and overall conclusion. However, items that have had the same tests completed and the same results observed may be combined into a single results section and overall conclusion. Inconclusive conclusions for any item shall have an explanation for that determination included within the report.

Mock reports are provided as a template for report formatting and guide to assist with the generation of Body Fluid Identification laboratory reports.

Mock #1

Mock #2

Mock #3

2.0 DNA Procedures

2.1 Biological Sample Handling

2.2 DNA Extraction

2.3 DNA Collection

2.4 Outsourcing DNA Casework

2.5 Internal Laboratory Control-Purchase, Preparation and Storage

2.6 DNA Case File

2.7 Familial Searching and Partial Matches

2.8 DNA Technical Leader Vacancy

2.9 Vacant

2.10 Interpretational Guidelines for AB 3500/3500xL STR Profiles-PowerPlex® Fusion

2.11 Interpretational Guidelines for AB 3500/3500xL STR Profiles- PowerPlex® Fusion and STRMix

2.12 DNA Extracts and Amplified Product

2.13 Vacant

2.14 DNA Report

2.15 Drying and re-Hydrating DNA Extracts or Liquid Samples

2.16 Administrative Review of Forensic DNA Casework Reports

2.17 DNA Specimen Entry into CODIS at LDIS-PowerPlex Fusion

2.18 Continuing Education

2.19 Plexor® HY with the Applied Biosystems 7500 Real-Time PCR System

2.20 Technical Review of DNA Casework

2.21 Vacant

2.22 Vacant

2.23 PowerPlex Y23 with AB 3500 Genetic Analyzer

2.24 PowerPlex™ Fusion with 3500/3500xL Genetic Analyzer

2.25 Vacant

2.1 Biological Sample Handling

2.1 Biological Sample Handling

2.1.1 Evidence Conservation

2.1.1.1

When examining evidence, a good faith effort will be made to ensure that a suspected stain or sample is not consumed in testing. A portion should be maintained for any potential re-testing.

2.1.1.2

If a sample or stain must be consumed in order to obtain a result, permission to consume the sample must be obtained from the submitting agency or Prosecutor.

2.1.2 Aseptic Technique and Contamination Control

2.1.2.1

All items used in the identification, transfer and isolation of samples must be sterile and/or free of contaminate DNA.

2.1.2.2

Laboratory coats must be worn at all times while in a Biology Unit laboratory examination area. Gloves and a face mask must be worn at all times while handling biological evidence or preparing reagents used in the analysis of biological evidence, or in the immediate vicinity of these activities. The use of a face mask is optional in the post-amplification room. Gloves must be changed frequently when handling different items of evidence. Every effort shall be made to minimize exposure between examination items (evidence items, reagents, plastic ware etc.) and laboratory staff members not actively engaged in biological analysis including minimizing foot traffic, casual conversations, time spent in analytical areas while not conducting analytical examinations and increasing the use of proper personal protective measures.

2.1.2.3

A fresh, sterile pipette tip must be used for each transfer of biological material or chemical to be used for analysis.

2.1.2.4

All analyses shall be performed on a clean work surface or disposable bench paper. The surface shall be cleaned with a 10% bleach solution or EPA registered sanitizer.

2.1.2.5

Scissors, tweezers, and other instruments used for examinations or analyses shall be cleaned with a 10% bleach solution, or EPA registered sanitizer, and rinsed with deionized water in between each sample. Items identified as single use shall be disposed of prior to examining next item.

2.1.2.6

The itemization, characterization and analysis of the unknown, or evidentiary items, and known samples shall be separated by time and/or space. The extraction of unknowns shall be initiated prior to known samples. Products of conception shall be processed with the unknown, or evidentiary, items. Reference DNA samples from children involved in relationship testing shall be processed with the known samples.

2.1.2.7

DNA laboratory areas shall be cleaned on a routine basis with a 10% bleach solution or EPA registered sanitizer. This includes laboratory surfaces, incubators and instruments, door handles and other areas that may be a source of contaminate DNA.

2.1.2.8

General facility cleanliness such as trash removal, biohazard disposal, sweeping, mopping and vacuuming will be the responsibility of the janitorial staff.

2.1.3 Documentation

2.1.3.1

A thorough record must be maintained on all examinations.

2.1.3.2

All analyses, results and appropriate controls must be documented.

2.1.4 Transfers

2.1.4.1

Transfer of Liquid Samples onto Bloodstain Cards
2.1.4.1.1

Samples to be transferred will be processed one at a time.
2.1.4.1.2

Label outer packaging and blood collection tube with laboratory number/item number (i.e. LS08-1234-1) and analyst initials.
2.1.4.1.3

Label bloodstain card as in 2.1.4.1.2.
2.1.4.1.4

Aliquot liquid blood onto card.
2.1.4.1.5

Before preparing next bloodstain card, visually confirm labeling in 2.1.4.1.2 matches stain card.

2.1.4.2

Transfer of Known or Evidentiary Samples for Extraction.
2.1.4.2.1

Samples to be transferred will be processed one at a time.
2.1.4.2.2

Evidentiary items will be processed before the knowns.
2.1.4.2.3

Label outer packaging with laboratory number/item number (i.e. LS08-1234-1) and analyst initials.
2.1.4.2.4

Label empty tube with DNA record/item number.
2.1.4.2.5

Transfer known or evidentiary samples to corresponding tube, using working rack if necessary.
2.1.4.2.6

After finishing the transfer, confirm that the labeled tube matches the outer packaging.
2.1.4.2.7

The known and evidentiary sample tubes will be place in separate racks for storage and/or further processing.

2.1.4.3

Transfer procedure for tube-to-tube and tube-to-concentrator:
2.1.4.3.1

Arrange samples in designated order keeping known samples and evidentiary samples in separate racks.
2.1.4.3.2

Label empty tube/concentrator with laboratory number/item number (i.e. LS08-1234-1) and analyst initials.
2.1.4.3.3

Place labeled empty tubes or concentrators in separate racks in same order as samples in step 2.1.4.3.1.
2.1.4.3.4

Remove sample to be transferred and the corresponding labeled new tube to a working rack and perform the transfer.
2.1.4.3.5

After finishing the DNA transfer, confirm that original tube and the transfer tube match each other.

2.1.4.4

Transfer procedure for tube to 96-well plate.
2.1.4.4.1

Arrange samples in designated order keeping known samples and evidentiary samples in separate racks.
2.1.4.4.2

Label 96-well plate with initials, date and procedural step (i.e. amplification of PP, amplification of CO, 310 plate PP, 310 plate CO etc.)
2.1.4.4.3

Remove sample to be transferred to well A1 of the 96-well plate to a working rack.
2.1.4.4.4

Perform the DNA transfer and place the tube in a new rack in the same position.
2.1.4.4.5

Continue transferring DNA in the manner outlined for well B1, C1, D1 etc.
2.1.4.4.6

When column 1 is complete, cap with strip caps.
2.1.4.4.7

Continue with the evidentiary items until completed and then continue with reference samples in a new column on the 96- well plate following the outlined procedure.
2.1.4.4.8

After finishing all DNA transfers, confirm that the tubes are arranged in the same order as transferred to the 96-well plate per the amplification worksheet.

2.1.4.5

Transfer procedure for 96-well plate to 96-well plate.
2.1.4.5.1

Briefly centrifuge source 96-well plate at low speed.
2.1.4.5.2

Remove one column of strip caps from source plate.
2.1.4.5.3

Remove samples from open column of source plate sequentially, one at a time, or with an 8 channel pipettor and transfer to destination 96-well plate.
2.1.4.5.3.1

Label destination 96-well plate with a unique identifier.
This could include a combination of the analyst initials, date and procedural step.
2.1.4.5.4

Place a NEW set of strip caps on the source and destination plate prior to beginning the next column of samples.
2.1.4.5.5

Complete the transfers as outlined for the remaining columns of samples.

2.1.5 PCR Testing

All PCR based operations will be maintained in separate dedicated rooms.

2.1.5.1

Pre-amplification laboratory
2.1.5.1.1

All reagents used to set up sample amplification will be maintained in the pre-Amplification laboratory. No amplified product will be introduced to this laboratory.
2.1.5.1.2

All reactions will be set up in PCR dead air containment hoods. The containment hoods should be sterilized using the onboard UV light for a minimum of 10 minutes before use and a minimum 10 minutes after each use.
2.1.5.1.3

The analyst will change gloves frequently to prevent casual transfer of sample.
2.1.5.1.4

Dedicated pipettes will be maintained in this area for reagent and sample transfer.
2.1.5.1.5

The area will be cleaned after each use and bench tops wiped down with 10% bleach solution.

2.1.5.2

Amplification Laboratory
2.1.5.2.1

All amplification will be performed in this room.
2.1.5.2.2

All allelic ladders and amplified product samples will be maintained in this room.
2.1.5.2.3

All amplified product will be maintained in this laboratory. No amplified product will be removed unless special precautions are taken.
2.1.5.2.4

Dedicated pipettes will be maintained in this laboratory for sample handling.
2.1.5.2.5

The analyst will change gloves frequently to prevent casual transfer of sample.
2.1.5.2.6

The area will be cleaned after each use and bench tops wiped down with 10% bleach solution.
2.1.5.2.7

Amplification sample trays will be soaked in bleach solution and rinsed with DI water before returning to the Pre-amplification laboratory.

2.1.6 Storage of Extracts

Pending further analysis, DNA extracts shall be stored for short term (overnight) at 4°C or for longer periods at -20°C.

2.1.7 PCR Product Transfer

Transfer of product may be deemed necessary from time to time (laboratory specific). Isolation vessels will be employed to prevent contamination of secondary environments. The containment vessels will be wiped down with 10% bleach solution before carrying the vessel to a new environment.

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2.2 DNA Extraction

2.2 DNA Extraction

2.2.1 Non-semen Samples

2.2.1.1 Place the sample into an extraction tube.

2.2.1.2 Add the following chemicals:

400 µL Stain Extraction Buffer (SEB)

20 µL Proteinase K

2.2.1.3 Vortex and microfuge briefly.

2.2.1.4 Incubate at 56°C for 2 hours or overnight.

2.2.1.5 If needed, place the sample material into a spin basket and insert into the corresponding tube. Otherwise, proceed to 2.2.1.6.

2.2.1.6 Microfuge for 5 minutes at 14,000 RCF.

2.2.1.7 If a spin basket was used, it should be removed and discarded at this point.

2.2.1.8 Add an equal volume of Phenol/Chloroform/Isoamyl Alcohol to the filtrate tube. This step must be carried out in a chemical fume hood.

2.2.1.9 Vortex vigorously until a milky emulsion is achieved in the tube. This step must be carried out in a chemical fume hood.

2.2.1.10 Microfuge for five minutes at 14,000 RCF.

2.2.1.11 Transfer the aqueous layer containing the DNA to corresponding tube.

2.2.1.12 At the analysts discretion, the aqueous layer may be reprocessed with the Phenol/Chloroform/Isoamyl Alcohol. Follow steps 2.2.1.8 through 2.2.1.11

2.2.1.13 Proceed to Biology Procedure manual section 2.3.1 (Microcon 100), 2.3.2 (Centricon 100) or 2.3.3 (Vivacon).

2.2.2 Differential (Semen Samples)

2.2.2.1 Place the sample into an extraction tube.

2.2.2.2 Add the following chemicals:

400 µL Tris-EDTA-NaCl (TNE)

25 µL 20% Sarkosyl

75 µL H_2O

5 µL Proteinase K

2.2.2.3 Vortex and microfuge briefly.

2.2.2.4 Incubate at 37°C for 2 hours.

2.2.2.5 Place the sample material into a spin basket and insert into the corresponding tube.

2.2.2.6 Microfuge for 5 minutes at 14,000 RCF.

2.2.2.7 Transfer the supernatant fluid to a new tube. This contains the female, or non-sperm fraction.

2.2.2.8 Discard the spin basket containing sample material.

2.2.2.9 Optional-repeat steps 2.2.2.2 through 2.2.2.6 for a cleaner fraction.

2.2.2.10 Store the female fraction at 4°C or -20°C until needed.

2.2.2.11 Wash the sperm pellet from Step 2.2.2.6 with TE $^{-4}$.

2.2.2.12 Spin for 5 minutes at 14,000 RCF and discard the supernatant. Repeat as necessary.

2.2.2.13 To the pellet add the following chemicals:

150 µL Tris-EDTA-NaCl (TNE)

50 µL 20% Sarkosyl

150 µL H_2O

10 µL Proteinase K

40 µL 0.39 Dithiothreitol (DTT)

2.2.2.14 Vortex and microfuge briefly.

2.2.2.15 Incubate at 37°C for 2 hours or overnight. This contains the male, or sperm fraction.

2.2.2.16 Extract both the fractions (male and female) by adding an equal volume of Phenol/Chloroform/Isoamyl Alcohol. This step must be carried out in a chemical fume hood.

2.2.2.17 Vortex vigorously until a milky emulsion is achieved in the tube. This step must be carried out in a chemical fume hood.

2.2.2.18 Microfuge for 5 minutes at 14,000 RCF.

2.2.2.19 Transfer the aqueous layer containing the DNA to the corresponding tube. Avoid removing denatured protein that collects at the interface.

2.2.2.20 At the analyst's discretion, the aqueous layer may be reprocessed with the Phenol/Chloroform/Isoamyl Alcohol. Follow steps 2.2.2.16 through 2.2.2.19.

2.2.2.21 Proceed to Biology Procedure manual section 2.3.1 (Microcon 100), 2.3.2 (Centricon 100) or 2.3.3 (Vivacon).

2.2.3 Non-Semen Samples with Tissue and Hair Extraction Kit

Note 1: This procedure shall be used in conjunction with Biology Procedure manual section 2.3.4 (Maxwell 16) for DNA collection. Items that are limited in nature and do not have sufficient biological material for additional DNA extraction attempts will not be extracted using this procedure.

Note 2: All components of the Tissue and Hair Extraction Kit must be stored at -20ºC prior to preparation of stock solutions.

2.2.3.1 Preparation of stock Proteinase K solution

2.2.3.1.1 Add 5.5 ml of Incubation Buffer to the bottle of lyophilized Proteinase K provided within the Tissue and Hair Extraction Kit. Swirl to dissolve. The final concentration will be 18 mg/ml.

2.2.3.1.2 Dispense the stock Proteinase K solution into aliquots that reflect usage and store at -20ºC for up to 1 year.

2.2.3.2 Preparation of 1M DTT

Dissolve 5 grams of DTT provided within the Tissue and Hair Extraction Kit in nuclease-free water so that the final volume is 32.4 ml. Dispense the DTT into smaller aliquots that reflect usage and store at -20ºC.

2.2.3.3 Preparation of Master Mix (Incubation Buffer/Proteinase K Solution)

2.2.3.3.1 The Master Mix must be prepared fresh for each set of extractions.

2.2.3.3.2 Combine the Incubation Buffer, 1M DTT and the stock Proteinase K solution in the proportions indicated below noting that each sample requires 480 µL:

Incubation Buffer	400 µL
1M DTT	40 µL
Stock Proteinase K solution	40 µL

Mix gently and use promptly.

2.2.3.4 Place the sample into an extraction tube and add 480 µL of Master Mix.

2.2.3.5 Vortex and microfuge briefly.

2.2.3.6 Incubate at 56ºC for approximately 1 hour.

2.2.3.7 Remove the samples from the incubator, vortex and microfuge briefly.

2.2.3.8 Add 400 µL of Lysis Buffer supplied with the Maxwell 16 DNA IQ Casework Extraction Kit specified in Biology Procedure manual section 2.3.4 to each sample. Vortex and microfuge briefly.

2.2.3.9 Transfer the cutting to a spin basket and return to the corresponding tube. Microfuge at approximately 14,000 RCF for 2 minutes. Discard spin basket containing sample cutting.

2.2.3.10 Proceed directly to Biology Procedure manual section 2.3.4 for DNA collection utilizing the Maxwell 16, DNA IQ Casework Sample Kit and Forensic LEV procedure.

2.2.4 Non-Semen samples using the QIASymphony collection method

Supplies:

Lyse and Spin Basket Kit: Catalog number 19597

QIASymphony DNA Investigator Kit (192): Catalog number 931436

2.2.4.1 Remove the QIAGEN spin basket from the individual wrapping and discard the unneeded tube.

2.2.4.2 Place sample into a 2mL QIAGEN spin basket microcentrifuge tube and label.

2.2.4.3 Prepare extraction master mix as outlined below:

ATL buffer 475µl per sample

Proteinase K 25µl per sample

NOTE: In order to account for pipette error add 2 extra samples per 10 samples extracted.

2.2.4.4 Vortex the master mix briefly and add 500µl to each sample.

2.2.4.5 Vortex each sample briefly.

2.2.4.6 Incubate the samples at 56°C for 1 hour vortexing the samples after 30 minutes.

2.2.4.7 Centrifuge the samples for 4 minutes at 14000 x g.

NOTE: Make sure all liquid has gone through the membrane.

2.2.4.8 DNA purification may be completed on the QIASymphony robotic platform or stored frozen indefinitely. Samples must be completely thawed and free from precipitates prior to DNA collection with the QIASymphony robotic platform.

2.3 DNA Collection

2.3 DNA Collection

2.3.1 Microcon 100 Microconcentrator

Available through Millipore (Catalog #42414 (500 pack), #42413 (100 pack))

2.3.1.1 Label the Microcon sample reservoir and supplied microfuge tube.

2.3.1.2 Insert sample reservoir into microfuge tube.

2.3.1.3 Rinse membrane with 100 µL TE $^{-4}$.

2.3.1.4 Microfuge at approximately 500 RCF for 5 minutes.

2.3.1.5 Pipette DNA sample into sample reservoir. Avoid touching the membrane with the pipette tip.

2.3.1.6 Microfuge at approximately 500 RCF for 15 minutes.

2.3.1.7 Remove filtrate from microfuge and transfer to a new, labeled 2.0 mL tube for temporary storage.

2.3.1.8 Wash DNA with 400 µL TE $^{-4}$.

2.3.1.9 Microfuge at approximately 500 RCF for 10 to 15 minutes.

2.3.1.10 Repeat steps 2.3.1.7 through 2.3.1.9 as necessary.

2.3.1.11 After final wash, membrane should appear moist, but without standing liquid on top. Continue microfuging until membrane has this appearance.

2.3.1.12 Add 16 µL TE $^{-4}$ to the sample reservoir.

2.3.1.13 Place the sample reservoir upside down into a new labeled microfuge tube.

2.3.1.14 Microfuge for 3 minutes at approximately 1000 RCF to transfer the collected DNA to the microfuge tube.

2.3.1.15 Record volume of DNA collected on the appropriate worksheet.

2.3.1.16 Retain filtrate washes until quantitation procedures are complete and amount of DNA is confirmed.

2.3.2 Vivacon 2 Centrifugal Concentrator (100,000 MWCO)

Available through Vivaproducts
(Catalog #VN02H242 (100 pack), #VN02H43 (500 pack))

2.3.2.1 At a minimum, label the sample reservoir and retentate vial with a unique identifier.

2.3.2.2 Insert sample reservoir into the filtrate vial. Always cover sample reservoir with retentate vial when centrifuging.

2.3.2.3 A membrane rinse may be utilized with approximately 500 µL TE $^{-4}$ followed by centrifugation at approximately 1,000 g-force using the Allegra XR-15 centrifuge with swinging bucket rotor for 5 minutes or at 2,500 g-force using the Eppendorf 5702 centrifuge with fixed rotor for 5 minutes.

2.3.2.4 Pipette 1 mL TE $^{-4}$ into sample reservoir

2.3.2.5 Pipette DNA sample into sample reservoir. Avoid touching the membrane with the pipette tip

2.3.2.6 Centrifuge at approximately 1,000 g-force using the Allegra XR-15 centrifuge with swinging bucket rotor for 10-20 minutes or at 2,500 g-force using the Eppendorf 5702 centrifuge with fixed angle rotor for approximately 15 minutes.

2.3.2.7.1 If additional washes are necessary, continue by adding 1 mL TE $^{-4}$ and centrifuge at approximately 1,000 g-force using the Allegra XR-15 centrifuge with swinging bucket rotor for 10-20 minutes or 2,500 g-force using the Eppendorf 5702 centrifuge with fixed angle rotor for approximately 15 minutes.

2.3.2.8 After the final wash, centrifuge until only a small amount of TE $^{-4}$ remains in the reservoir. There should be approximately 20 - 100µL remaining

2.3.2.9 Place the sample reservoir upside down into the retentate vial

2.3.2.10 Centrifuge for 3 minutes at approximately 1,500 g-force using the Allegra XR-15 centrifuge with swinging bucket rotor or at 2,500 g-force using the Eppendorf 5702 with fixed rotor for 2 minutes to transfer the collected DNA to the sample reservoir. A spacer insert may need to be used during this step when using the Eppendorf 5702 with fixed rotor.

2.3.2.11 Transfer recovered DNA to a new, labeled microfuge tube or other appropriate storage vessel to store for future use

2.3.2.12 Record volume of DNA collected on the appropriate worksheet

2.3.2.13 Retain filtrate washes until quantitation procedures are complete and amount of DNA is confirmed

2.3.3 DNA Collection using the Maxwell 16 DNA IQ Casework Sample Kit with the Maxwell 16 LEV Instrument

DNA IQ Casework Sample Kit for Maxwell 16 (Promega AS1210 or AS1240). Contents include Elution Tubes, Elution Buffer, Lysis Buffer, LEV Plungers and DNA IQ Casework Sample Cartridges. The kit components can be stored at 20-25ºC.

All required information shall be recorded on the FS-63.

2.3.3.1 Wipe the surface of the magnetic rod assembly, plunger bar, inside platform and the outside of the instrument using a cloth dampened with 70% Ethanol or de-ionized water. Ensure that all surfaces are dry prior to proceeding to the next step.

2.3.3.2 Place the number of cartridges to be used into the Maxwell 16 LEV Cartridge Rack with the tube holder facing the numbered side of the rack. Hold the cartridge firmly and remove the seal. If collecting less than the 16 maximum samples, evenly space the cartridges outwards from the center of the rack.

2.3.3.3 Visually inspect the plastic plunger sleeve for any malformations. If plastic plunger sleeves with malformations are observed, remove from circulation. Place one plunger into well #8 of each cartridge.

2.3.3.4 Place labeled 0.5 ml Elution Tubes at the front of each cartridge. Add 25-50 µL of Elution Buffer to each Elution Tube.

2.3.3.5 Transfer the extracted sample from Biology Procedure manual section 2.2.3 to well #1 of the cartridge corresponding to the appropriate sample.

2.3.3.6 Turn the Maxwell 16 Instrument on. Scroll to "Run" on the Menu screen and press the "Run/Stop" button to start the method.

2.3.3.7 Open the door when prompted on the LCD display. Press the "Run/Stop" button to extend the platform.

2.3.3.8 Transfer the Maxwell 16 LEV Cartridge Rack onto the Maxwell 16 platform with the tube holders closest to the door. Ensure that the cartridge rack is completely level. Press the "Run/Stop" button. The platform will retract, close the door.

2.3.3.9 When the purification is complete, the LCD screen will display a message that the method has ended. At this point, open the door. The plungers should be located in well #8. Press the "Run/Stop" button to extend the platform.

2.3.3.10 Remove the Elution Tubes from the heated elution tube slots and close the top of each tube. Samples may be stored at 4ºC overnight or -20 ºC for extended periods of time.

2.3.3.11 Wipe the surface of the magnetic rod assembly, plunger bar, inside platform and the outside of the instrument using a cloth dampened with 70% Ethanol or de-ionized water.

2.3.4 DNA Collection using the QIASymphony Robotic Platform

Supplies:

Elution tubes: Sarstedt 1.5ml Ref. 72.607

Elution tube screw caps: Startedt Ref. 65.716

8-well sample preparation cartridges: Catalog number 997002

8-rod covers: Catalog number 997004

QIASymphony o-ring set: Catalog number 9019168

200uL Filter Tips: Catalog number 990332

1500uL Filter Tips: Catalog number 997024

Topelute fluid 60/60KG: Catalog number 1055628

The QIASymphony may only be used for DNA lysates that were created using procedure 2.2.4.

2.3.4.1 Cut the lid apart from the tube and remove the lid and the filtration column when ready to place each sample into the sample tube carrier.

2.3.4.2 Before starting the lysis and filtration procedure, turn the QIASymphony on using the power button on the lower left hand side of the instrument.

2.3.4.3 Tap on the “Login” button on the upper right hand corner of the touch screen.

Enter Login: Supervisor

Enter Password: dna123

2.3.4.4 Starting the Wizard: Go to the “sample preparation” tab and from the sample preparation screen choose “Wizard”.

2.3.4.5 Select Assay Control Sets and Number of Samples Screen: Under the heading “Available Assay Control Sets”, select “Investigator.”

2.3.4.6 Use the arrow at the bottom of the list to scroll down to the protocol “CW 500 ADV HE V8.” To select it, click on the protocol and then tap on the arrow pointing to the right next to the protocol list.

2.3.4.7 Under the heading “Selected Assay Control Sets/ Number of Samples” tap on the white box with the number 1 in it. Enter in the number of samples.

2.3.4.8 Tap “Next”.

2.3.4.9 If any unit boxes are full place a lid on the container, then discard it in the biohazard bin. Replace them with empty containers for the used rod covers and sample prep cartridges.

2.3.4.10 Check the liquid waste level and dispose of any liquid in the designated waste container.

2.3.4.11 Check to make sure the tip shoot and the tip park station are in place.

2.3.4.12 Check to make sure the tip disposal bag is not full. If it's full, dispose of that waste in the biohazard bin and replace with a new bag.

2.3.4.13 Close the drawer and tap "Next" on the lower right of the screen. An inventory scan will automatically occur.

2.3.4.14 Place a lid on the 1.5mL screw cap tubes and then label the 1.5mL screw cap elution tubes with the sample names.

2.3.4.15 Remove the tube caps and place on and cover them up with a Kimwipe until ready to be used again. Place the tubes in the elution rack in the order that corresponds to the order of the sample tube carrier.

NOTE: The elution rack is ordered going left to right down the columns.

1	5	9	13	17	21
2	6	10	14	18	22
3	7	11	15	19	23
4	8	12	16	20	24

2.3.4.16 Place the elution rack in a slot of the eluate drawer starting with slot 2.

NOTE: Do not use the first slot which is the cooling slot.

2.3.4.17 Select the elution rack number being used on the left side of the screen.

2.3.4.18 In the center of the screen under "Available Rack Types," tap on the "Tube 1.5mL" and then tap on "SAR #72.607 T1.5 Screw."

2.3.4.19 Select the rack on the left of the screen and tap on "Rack ID"on the top right of the screen. Enter the name of the rack and touch "Ok."

2.3.4.20 Close the drawer and tap "Next" on the lower right of the screen. An inventory scan will automatically occur.

NOTE: When the eluate cooling error appears, click "Yes" both times.

2.3.4.21 Touch the "Bottle ID" button on the screen and select the text area to scan the barcode on the TopElute bottle. Use the scanner on the right side of the cabinet below the QIAsymphony. Place the TopElute bottle in the round opening at the back of the drawer.

2.3.4.22 Unopened reagent cartridge: Pull out the reagent cartridge, enzyme rack, carrier RNA, and buffer ATE from the DNA Investigator Kit kept at room temperature.

2.3.4.23 Add 1.6mL of buffer ATE into the tube of RNA. Then, vortex and quick spin.

2.3.4.24 Tranfer 400µl of RNA into positions 1 and 2 of the enzyme rack.

2.3.4.25 Add 1.2mL of additional buffer ATE to each tube on the enzyme rack. Mix it with a pipette.

2.3.4.26 Place the piercing lid on top of the cartridge.

2.3.4.27 Remove the magnetic bead trough and vortex for 3 minutes and then place it back where it was found and remove the cover.

2.3.4.28 Place the reagent cartridge on the gray reagent cartridge holder.

2.3.4.29 Already opened reagent cartridge: Pull out the reagent cartridge kept at room temperature and the enzyme rack kept in the fridge.

2.3.4.30 Remove the magnetic bead trough and vortex for 3 minutes and then place it back where it was found.

2.3.4.31 Remove the plastic seals from the reagent wells and the magnetic bead trough.

2.3.4.32 Remove the enzyme tube caps and place them below where the enzyme rack sits on the reagent cartridge before attaching the enzyme rack.

2.3.4.33 Place the reagent cartridge in the reagents and consumables drawer. The enzyme rack will be toward the back of the slot that the cartridge sits in.

2.3.4.34 Tap "Next" on the lower right of the screen.

2.3.4.35 At the top of the screen there is a table which tells you how much of each item will be needed to run the number of samples being run.

2.3.4.36 Load the tips.

200µl tips are in the 4 slots toward the back of the drawer.

1500µl tips are in the other 14 slots that are toward the front of the drawer.

2.3.4.37 Load rod covers and sample prep cartridges.

Sample prep cartridges are loaded in the back 3 slots of the drawer.

Rod covers are loaded in 1 slot toward the front of the drawer.

NOTE: These should be in proportion of 5 cartridges to 1 rod cover.

2.3.4.38 Close the drawer and tap “Next” on the lower right of the screen and wait for the inventory scan to complete.

2.3.4.39 Touch “Use tube carrier” on the left side of the screen

2.3.4.40 Tap “Next” on the lower right of the screen.

2.3.4.41 Load the samples into the sample tube carrier with the caps and spin baskets removed.

2.3.4.42 Load the sample tube carrier into the drawer.

Slide the tube carrier to the line and stop. Wait for the laser to turn on and then push the rack in one fluid motion

NOTE: On the screen the tube carrier slot will be highlighted yellow if waiting for the carrier and will turn green when it has been loaded successfully.

2.3.4.43 Tap “Next.”

2.3.4.44 Under the “Sample Tube Selection” select all samples by tapping the gray area with numbers above each column.

2.3.4.45 Under “Inserts/Sample Tubes” on the right of the screen tap on “Tube insert 3B” and then tap “QIA #19201 Collection.”

2.3.4.46 Tap the “ID/Type” button toward the top right of the screen.

2.3.4.47 Click on each individual sample and tap “Sample ID” on the right side of the screen.

2.3.4.48 Type in the sample name and hit “Ok.”

2.3.4.49 After all samples have been named, tap “Next” on the lower right of the screen.

2.3.4.50 Under the heading “Assign Assay Control Sets to Samples and/or Check Automatic Work List Assignments” highlight all samples.

2.3.4.51 Under “Application/ACS” choose “CW 500 ADV HE V8” and tap “Next.”

2.3.4.52 Tap on the elution rack on the left side of the screen and under the “Eluate volume” tab choose 40µl.

2.3.4.53 Tap “Finish” and say yes to the eluate cooling error.

2.3.4.54 Select “Run” on the sample prep screen to start the batch. Tap “Yes” when the eluate cooling error appears.

2.3.4.55 Before starting the run this will calculate the number of samples that can be run with the amount of consumables and reagents that are loaded in the drawer.

2.3.4.56 Go to the R+C button on the bottom of the screen.

2.3.4.57 Tap sample calculation at the top right of the screen.

2.3.4.58 Under “Application/ACS” choose “CW 500 ADV HE V8” and wait for the calculation to occur. Tap “Ok” to return to the sample prep screen.

2.3.4.59 Remove the elution rack and put the caps on the tubes and then remove them from the rack.

2.3.4.60 Remove the sample tube carrier and throw away the empty sample tubes in the biohazard bin.

2.3.4.61 Remove the reagent cartridge from the drawer. Take the enzyme rack off the reagent cartridge and place the caps back on. Store it in the fridge.

2.3.4.62 Seal the reagent wells and magnetic bead trough with the reusable plastic seal set included in the DNA Investigator kit. Label the plastic seals with the corresponding reagent number and mark the date the cartridge was opened. Store it at room temperature.

NOTE: The cartridge expires two weeks after it has been opened.

2.3.4.63 Remove the TopElute and place the lid back on. Store it at room temperature.

2.3.4.64 Remove any unused tips for later use and throw away any empty tip racks.

2.3.4.65 Place covers on unused rod covers and sample prep cartridges and remove for later use. Store the empty unit boxes for use as waste containers.

2.3.4.66 Remove any full waste containers, place a lid on them and dispose of them in the biohazard bin.

2.3.4.67 Empty the liquid waste into the designated container.

2.3.4.68 Wipe down instrument using the “Super Sani-cloth Germicidal Disposable Wipe.”

2.3.4.69 UV the QIAsymphony for 60 minutes. From the main menu, go to the Maintenance screen. Press the “Start UV Light” button, change time to 60 minutes and press “Enter” to start the UV procedure.

2.4 Outsourcing DNA Casework

2.4 Outsourcing DNA Casework

In the event that use of a vendor laboratory is necessary, the following procedures shall be followed for the laboratory selection, casework initiation and technical review of data and reports generated.

2.4.1

Vendor laboratories utilized by the Michigan State Police and local law enforcement agencies shall meet the following criteria, at a minimum:

2.4.1.1

Be currently accredited by an NDIS-approved accrediting body

2.4.1.2

Comply with the FBI's Quality Assurance Standards for Forensic DNA Testing Laboratories

2.4.1.3

Utilize the same Platform, Technology and amplification kit(s) currently in use by the Michigan State Police

2.4.2 On-Site Visits

An on site visit of the vendor laboratory must be conducted at the onset of a multi-year contract period by the DNA Technical Leader or his/her appointee. Subsequent on site visits must be conducted annually. With the exception of the initial on site visit, a review of an on site visit conducted by another NDIS-participating laboratory may be acceptable. All on site visits must be documented and approved by the DNA Technical Leader.

2.4.2.1

For on site visits conducted by Michigan State Police personnel, it must include a laboratory tour and review of recently approved validations, newly trained personnel qualifications, procedure manual, quality manual, technical specifications, audits/inspections and corrective action reports.

2.4.3 Case Reviews

DNA cases that have been outsourced to a vendor laboratory and were submitted by the Forensic Science Division must go through a technical and administrative review process. DNA cases that have been directly outsourced to an MSP-accepted vendor laboratory by a local law enforcement agency resulting in a DNA profile eligible for CODIS entry as indicated by the vendor laboratory must go through a technical and administrative review process. For cases outsourced directly by local law enforcement agencies, the Forensic Science Division may opt to utilize contract personnel for the technical review of those cases requiring a CODIS entry. When contract personnel are utilized for the technical review, a standardized technical review form shall be utilized by both the contract personnel conducting the technical review and the FSD personnel completing the CODIS entry, when applicable, to document the review and which personnel are responsible for each area of the complete review process. For both MSP-reviewed and contractor/MSP-reviewed outsourced cases, the technical review shall consist of:

2.4.3.1

A review of the DNA types reported to verify that they are supported by raw and/or analyzed data.

2.4.3.2

A review of all associated controls, size standards and allelic ladders to ensure that they meet expected results.

2.4.3.3

A review of the report generated by the vendor laboratory to verify the conclusions are supported by the data provided, that each sample submitted is addressed in the report and the data reported meets the technical specifications approved by the DNA Technical Leader for entry into CODIS.
2.4.3.3.1

CODIS entries for outsourced casework where the technical review is completed by MSP contract personnel shall be entered, verified and uploaded by the MSP LDIS laboratory. Verification of the CODIS entry shall be documented on the technical review form.

2.5 Internal Laboratory Control-Purchase, Preparation and Storage

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2.5.1 Internal Laboratory Control Purchase

The Internal Laboratory Control may be purchased from Golden West Biologicals, Inc. It should be a single source male blood sample freshly collected in an EDTA tube or bag.

2.5.2 Internal Laboratory Control Preparation

Upon arrival at the laboratory, the blood sample shall be stored refrigerated until it is checked and aliquotted. The blood sample shall be given a lot number (i.e. GWB ILC and the date of receipt) for record keeping purposes.

The blood sample shall be aliquotted onto a suitable substrate for long term storage and use such as filter paper or FTA paper. The aliquot volume should be in accordance with usage to minimize the possiblity of repeated sampling or potential degradation.

Once aliquotted, the blood sample shall be checked against the NIST SRM 2391C and 2395 by analyzing in parallel with the SRM samples. All relevant worksheets and data shall be forwarded to the DNA Technical Leader/Biology Program Coordinator. Approval of the Internal Laboratory Control as a NIST-traceable SRM shall be documented by the DNA Technical Leader/Biology Program Coordinator.

2.5.3 Internal Laboratory Control Storage

The Internal Laboratory Control shall be stored either frozen or refrigerated. It may be stored at room temperature while in use.

2.6 DNA Case File

2.6 DNA Case File

2.6.1 The following documentation shall be included in each DNA case file:

2.6.1.1

DNA Extraction, Quantitation and Amplification worksheets

2.6.1.2

Sample Dilution worksheet(s)

2.6.1.3

Mixture Interpretation worksheet as required by procedure

2.6.1.4

.aan file from Plexor HY quantitation

2.6.1.5

Plexor HY software Forensic Report

2.6.1.6

FL and Y Plexor HY standard curve data

2.6.1.7

CODIS Specimen Detail Report (if applicable) with a screen capture of the Moderate Match Estimation include in the file (where applicable).

2.6.1.8

PopStats calculations (if applicable)

2.6.1.9

Electropherograms of positive controls

2.6.1.10

Electropherograms of negative controls that meet the most restrictive volume and amplification conditions as compared to case samples

2.6.1.11

Electropherograms of a representative ladder from each Genetic Analyzer run

2.6.1.12

Communication documents (if applicable)

2.6.1.13

Electropherograms from each item analyzed and each amplification for each item shall be included within the case file. The electropherogram utilized for interpretation shall be clearly marked.

2.6.2 Optional

A separate GMID project for each case may be created and include the .fsa and .ser files in the DNA case file Object Repository.

2.7 Familial Searching and Partial Matches

2.7.1 Familial Search Policy

2.7.2 Familial Search Procedure

2.7.3 Partial Match Policy

2.7.4 Partial Match Procedure

2.7.1 Familial Search Policy

2.7.1.1 Introduction

The Michigan State Police has developed a DNA Familial Search policy that may result in investigative information provided to law enforcement officials in unsolved cases where all other investigative leads have been exhausted. The policy and procedures were developed keeping privacy concerns in mind while at the same time providing information that may be useful in solving a violent offense.

2.7.1.2 Background

Michigan's DNA Database was established under the authority of the DNA Identification Profiling System Act, Act 250 of 1990. The purpose of the Act is:

"to provide for a DNA identification profiling system; to provide for the collection of samples from certain prisoners, convicted offenders, and juvenile offenders and the analysis of those samples; and to prescribe the powers and duties of certain state departments and county agencies."

Additional provisions are located in The Michigan Penal Code, Act 328 of 1931 which further allow for the collection of DNA samples if the *"individ*

ual is arrested for a violent felony as that term is defined in section 36 of the corrections code of 1953, 1953 PA 232, MCL 791.236."

A familial search is a deliberate search of the Michigan DNA database for biologically-related relatives (siblings, parents, and children) of a contributor of an evidentiary profile from crime scene evidence. This search is performed with specialized software designed and validated for such purpose. The information that may develop from a familial search and ultimately be provided to law enforcement will be the name(s) of an individual(s) in Michigan's DNA database who may be closely related to the person whose DNA was identified on crime scene evidence.

A match is determined if a profile in the database is essentially the same as the profile identified on crime scene evidence. It is possible for the crime scene profile and a profile in the database to have a strong similarity between them, but not match completely. Sometimes these similarities are so strong that they may be biologically related to each other. With advances in DNA technology, lineage DNA analyses can now be conducted by the Michigan State Police to provide additional information as to the likelihood that two DNA profiles may be related.

The process associated with familial DNA searching requires special DNA testing and, possibly, a law enforcement investigation of the potential relative. Accordingly, policy on case acceptance for this process was developed taking into consideration both privacy concerns and agency resources, recognizing that it may provide information useful for identifying the perpetrator of a violent crime against a person, thus preventing further victimization.

2.7.1.3 Searching Criteria

Familial searching is considered the last resort in the use of the Michigan DNA database when attempting to identify the perpetrator of an unsolved crime. A familial search of the convicted offender and arrestee indices of the Michigan DNA Database, using special search software designed and validated as effective and accurate for this purpose, may be conducted at the directions of the Director of the Forensic Science Division and the Director of the Biometrics and Identification Division of the Michigan State Police in a case in which all of the following criteria are satisfied:

1. the request for the familial DNA search in a particular case is made by the chief law enforcement officer of the investigating law enforcement agency in writing;
2. the case involves an active investigation of an unsolved violent crime against a person or there are critical public safety implications;
3. other investigative leads have been exhausted and critical public safety concerns remain;
4. prior analysis of crime scene evidence has yielded a single-source DNA profile(s) without reasonable potential for allelic drop-out or drop-in or an unambiguous deduced single source profile from a DNA mixture which when traditionally searched against other databases at the state or national level has not developed any useful results;
5. the evidence exhibits a DNA profile of at least the core thirteen genetic markers accepted by the FBI (partial DNA typing results will be considered on a case by case basis);
6. the Director of FSD, Director of BID, FSD Biology Program Coordinator, CODIS State Administrator, requesting chief law enforcement officer, Prosecuting Attorney for the investigating jurisdiction, and any other personnel deemed necessary shall have conferred regarding the request, the case criteria, and the use of any search results; and
7. the chief law enforcement officer of the investigating law enforcement agency and the Prosecuting Attorney for the investigating jurisdiction shall agree in writing that the aforementioned criteria have been satisfied and commit to further investigation if potentially related individuals are identified.

If all case criteria are satisfied, the Directors of BID and FSD may approve the investigating agency's request that a familial search of the Michigan DNA database be performed. The search will be conducted in conformance with departmental scientific protocols.

2.7.2 Familial Search Procedure

2.7.2.1 Process

When invoked, the objective of this procedure is to obtain a candidate convicted offender or arrestee, hereafter referred to as an offender, or limited list of candidates, who may be closely related to the true perpetrator of a crime. As part of this process, the initial candidate list of offender DNA samples will be further filtered to include male offenders only. They will be analyzed for development of a Y-STR haplotype. The samples to be tested are selected by priority based primarily on kinship indices and an evaluation of the numbers of shared alleles. Any offenders not eliminated by the Y-STR haplotype comparison could be patrilineally related to the true perpetrator and will be candidates for further investigation and consideration as potential genetic relatives of the true perpetrator. This process is designed to provide the most useful investigative lead(s) while limiting the number of potential contacts with individuals not related to the perpetrator.

2.7.2.2 Confidentiality

Beyond the rigorous restrictions already imposed on Michigan DNA Database employees concerning disclosure of information, the results of the familial search conducted by the CODIS Unit and subsequent Y-STR haplotyping, if any, will be released only to the Director of the Forensic Science Division, Director of the Biometrics and Identification Division, Biology Program Coordinator and State CODIS Administrator herein referred to as the Familial Search Committee (FSC). Any wider distribution of results or data will occur only at the direction of the FSC. This precludes direct contact with the submitting laboratory by the CODIS Unit, in contrast to routine CODIS searching and operations. Since CODIS autosearch software is not utilized in the familial search process there is no automatic notification of the LDIS Laboratory of the results of the familial search. Direct contact with law enforcement or the prosecution will always be made by the FSC as opposed to the usual involvement of the CODIS Unit. The FSC generally retains the authority to release any familial search information to client groups in accordance with policy.

2.7.2.3 Considerations

The chance of success in identifying a close genetic relative of a perpetrator in the existing offender database is affected by these practical considerations:

1. There may be no such genetic relative in the database at the time of the search.
2. Estimates of the likelihood of familial relationships are made based on the sharing of STR alleles and the rarity of those alleles.
3. The search for genetic relatives is practical only for first degree relatives (i.e. full siblings that share both parents and parent/child relationships).
4. Information for female candidate results may be limited as Y-STRs are of no value.

In contrast to the identification of a putative perpetrator (or an identical twin) as a result of a confirmed offender hit, the output of a familial search can only indicate whether a statistically significant potential exists that an offender in the database may be a relative of the true putative perpetrator, based on the specific forensic unknown DNA profile submitted. Since multiple offender profiles may yield similar statistical results for kinship or allele sharing when compared to the forensic unknown STR profiles, further extensive investigation may be necessary to confirm the existence of relatives to the offender identified via familial search, as well as to determine whether any of the offender's relatives could have committed the crime. In any case, the results represent another form of an investigative lead in the investigation, rather than a more direct association or identification of an offender.

2.7.2.4 Familial Search Requests

All Familial Search requests shall be in writing and authored by the chief law enforcement officer of the investigating agency. The written requests shall be submitted to the Director of the Biometrics and Identification Division and Director of the Forensic Science Division. The request will be forwarded to MSP District Detective Lieutenant for investigative evaluation and follow-up with lead investigator of the investigative law enforcement agency. The request will also be forwarded to FSD's Biology Technical Leader and BID's State CODIS Administrator for evaluation of the quality and completeness of the DNA analysis. The MSP District Detective Lieutenant, Biology Technical Leader and State CODIS Administrator will report to the Directors of the Forensic Science Division and Biometrics and Identification Division indicating whether the request meets the Familial Search policy as outlined in 2.7.2.5. The Directors of the Forensic Science Division and Biometrics and Identification Division shall determine if the Familial Search request is granted.

2.7.2.5 Required Elements Prior to Familial Searching

These critical items must be complete and/or confirmed prior to initiation of this procedure:

1. That the evidentiary DNA profile is from a case having significant public safety concern and the familial search result is critical to advancing the investigation;
2. That the request from the investigating law enforcement agency includes a case summary and a CODIS specimen identifier;
3. That the investigating law enforcement agency and/or the Prosecuting Attorney agree to meet jointly with the MSP to discuss the meaning of the search results and to abide by any and all MSP policies or procedures related to familial searching;
4. That the lead investigator and prosecuting attorney assigned to the case has received MSP-approved training in the use of DNA familial search evidence;
5. That standard investigative leads have been exhausted, or a specific exception is articulated;
6. That the agency agrees to further investigate the case after MSP releases the identifying information to the requesting agency;
7. The requesting agency will have successfully determined the Y-STR type of the forensic unknown using an MSP-approved Y-STR amplification kit;
8. The requesting agency will have considered, attempted, or succeeded in obtaining single source DNA profile at all of the CODIS core 13 loci of the forensic unknown with an STR amplification kit approved by MSP.

2.7.2.6 Preliminary Standard Search

Upon receipt of direction and authorization from the Familial Search Committee (FSC), the CODIS Unit will receive the forensic unknown DNA profile(s) of interest and the associated case file.

- To confirm that a direct match to a perpetrator is not present, a preliminary, moderate stringency, manual or "keyboard" search, with routine Michigan SDIS search settings, will be conducted of the forensic unknown against the offender and forensic indexes in the database immediately prior to initiating any familial search. The negative state match detail report will be retained in the file.
- In the event of an offender hit, standard confirmatory processing will be initiated, the familial search process will be terminated and the FSC immediately notified.
- If a "partial match" is obtained, it will be noted and the state match detail report will be retained. The specimen involved will be considered for inclusion in the Y-STR processing list generated later at the discretion of the FSC.

2.7.2.7 12 High + 1 Mismatch Search* *

- As an additional quality assurance step, a search of the forensic unknown index will be conducted at high stringency at twelve loci with one mismatch allowed. Any resultant candidate matches under these search conditions will be documented and evaluated as potential offender hits in consultation with the FSC.
- In the event of an offender hit, the standard confirmatory process will be initiated, the familial search process will be terminated and the FSC immediately notified.

2.7.2.8 Conducting a familial search

A familial search will be initiated by the CODIS State Administrator and/or Biology Technical Leader using the MPKin^{TM} FS Edition software and will result in a list of candidate offenders. Females will be removed from the candidate list based upon Amelogenin results. The thresholds utilized in the software for the Kinship Index and Identity By State (IBS or shared alleles) will initially begin at 21 IBS and a KI of 100,000. The IBS and KI parameters will be reduced in a step-wise fashion until a sufficient number of candidates or 15 IBS/KI 1,000 are achieved. Y-STR typing of the selected candidate samples will be performed by the MSP using validated Y-STR methods on the top 80 parent:child candidates and the top 80 distinct full sibling candidates. Less than 160 candidates will be tested with Y-STRs if a fewer candidates are identified. Upon completion of the familial search, the .xml file containing the offender DNA profiles shall be deleted from the MPKin^{TM} FS Edition workstation.

The Y-STR haplotype of the target forensic unknown is compared against the set of Y-STR haplotypes obtained from the candidate offender samples identified for testing. The presence or absence of concordant offender Y-STR types is evaluated using standard interpretation guideline by the FSD Biology Program Coordinator and documented in the case file.

2.7.2.9 Reporting Results

The FSD Biology Technical Leader will notify the Directors of the Forensic Science Division and Biometrics and Identification Division of the existence or absence of offender Y-STR haplotypes concordant with the Y-STR haplotype of the target forensic unknown.

Where a potential investigative lead exists, the Directors will request the MSP District Detective Lieutenant to initiate a background investigation on each candidate to determine whether that candidate can be eliminated by historical facts, relationships or circumstances as being a potential relative of the true perpetrator. This investigation will include an evaluation of the fingerprint submitted on the DNA Database collection card, if

available. Upon return of the background investigation results to the Directors, they will meet and confer. Unless there is a reason not to do so, the Directors will authorize the release to the requesting agency the name(s) of the relevant offender(s) that may be possible relative(s) of the perpetrator through a laboratory report. The report will include identifying information of any individual having sufficient DNA markers in common with the DNA offender profile, to include the name, SID, and date of birth for each individual. The report will include the following statement:

"This information is for law enforcement investigatory purposes only. It is not a statement of identity. The release of the DNA profiles to non-law enforcement agencies/personnel is a violation of both state and federal statutes."

Where a potential offender with concordant Y-STR haplotype does not exist, the Directors will confer and request the Biology Technical Leader to author a laboratory report to the investigating agency communicating the negative results of the familial search.

2.7.2.10 MSP or Investigating Agency Follow-up

After receiving the identifying information determined by the familial search, the investigating law enforcement agency agrees to abide by the following policies, procedures and requirements:

1. The MSP or the investigating agency will conduct an initial review of the familial search results to determine individuals or families of immediate interest to the investigation.
2. When an individual is identified through the familial search, the investigating law enforcement agency shall investigate whether the identified individual is related to the DNA database offender. As applicable, the investigator may construct a "family tree" of male relatives connected through the DNA and passed through the y-chromosome.
3. To determine possible familial relationships, the investigating law enforcement agency, with assistance from MSP, as needed, shall conduct a full background check of the identified individual and family members, including use of the following sources, as available and applicable: 1) CCIC/NCIC criminal history checks; 2) Inmate profiles from MDOC; 3) Visitor logs from MDOC; 4) Pre-sentence investigation reports; 5) Jail records including visitor logs and telephone logs; 6) Court records searches; 7) Public records searches; 8) State vital records and 9) Other public resources.
4. Following a thorough records investigation of the individual or individuals identified through the DNA familial search as related to the offender, the investigating law enforcement agency shall examine the investigative records of the subject crime and determine whether this individual is, or these individuals are, possible suspect(s). Investigative steps and resources that could be utilized include: 1) Surveillance data; 2) DNA samples obtained surreptitiously; 3) Work or employment background; 4) Adult and juvenile criminal histories; 5) Motor vehicle records (driver's license, ID card, vehicle registration); 6) Housing records; 7) Financial searches and 8) Additional interviews or re-interviews of informants, witnesses, or victims.
5. Based upon information obtained through the investigation, the investigating law enforcement agency shall legally obtain a DNA sample from the identified suspect. Use of familial DNA alone shall not be the sole basis upon which an investigator requests the known sample for comparison. Additional evidence must be demonstrated to support the petition for a court order.
6. The sample obtained from the suspect will be typed by the MSP and compared with the forensic profile. MSP will provide the results of this comparison to the investigating law enforcement agency through a standard laboratory report.
7. Absent exigent circumstances, family members and relatives should only be contacted after initial investigative steps have been taken during the investigative process, to include first obtaining information from public and law enforcement authorized databases. Care should be taken to ensure consideration of potential family issues before contacting family members. Potential issues constituting reasons for delaying contact with family members include: 1) The possibility that a father is not aware of the existence of an offspring (the "unknown child" issue); 2) The possibility that a family might have assumed a child's father is someone else (the "misbelieved paternity") and 3) The existence of other possible family privacy concerns.

2.7.2.11 Documentation of Familial Search Process, Results and Communications

A complete case file will be maintained which contains all information, examination documentation, communication, meeting notes, etc. related to the familial search request. A new laboratory number will be pulled for each familial search. The documentation will also include a list of the specimen identifiers of the candidate offenders' that will be retained and included in the appropriate file associated with the requested search.

2.7.2.12 Repeat requests

Where no appropriate investigative leads are identified, the investigating agency may re-request a familial search every 6 months. The new search request will be evaluated using the same criteria for an initial search request. Subsequent familial searching for the same evidentiary profile will be logged as a new submission under the original laboratory number.

2.7.3 Partial Match Policy

2.7.3 Partial Match Policy

2.7.3.1 Introduction

The Michigan State Police has a policy for handling and reporting results of partial matches that occur fortuitously during standard searches of the CODIS database. This policy is intended to cover matches that occur at the SDIS level or matches that occur at the NDIS level and are requested in accordance with NDIS procedures.

2.7.3.2 Background

A partial match is the product of a routine database search in which a candidate offender profile is not identical to the forensic profile, but has a high number of similarities between the two profiles indicating a possible biological relationship between them.

Because of the independent assortment of alleles inherited from biological parents by human offspring and siblings, it is expected that the DNA profiles from genetically related persons will share some but not all alleles at the examined forensic STR loci. In the case of identical twins sharing is complete. The standard CODIS search employed at Michigan SDIS uses the moderate stringency comparison to allow for the comparison of an offender with a crime scene DNA profile that may be incomplete or a mixture. Candidate matches can occur using these search criteria that are not direct matches, but which may be to a potential relative of the true perpetrator. These candidate matches are classified as "partial matches".

2.7.3.3 Search Criteria

For partial matches, the name of the offender may be released to the investigating agency if the MSP procedure has been followed and all of the following conditions are met:

1. The crime scene DNA profile is a single-source profile or an unambiguous major profile from a mixture.
2. The case is unsolved and all investigative leads have been exhausted.
3. A commitment is made by the investigating agency and the Prosecuting Attorney to further investigate the case if the name of the potentially related offender is eventually released.
4. Y-STR typing of the same crime scene evidence that yielded the submitted forensic unknown profile is completed by the investigating agency and is concordant with the offender's Y-STR haplotype obtained by MSP.
5. If the Y-STR haplotypes have been determined to be consistent, MSP will review non-forensic information in order to identify additional evidence bearing on relatedness, if available.
6. The Familial Search Committee (FSC) will discuss the case with the investigating agency, the local laboratory, and the Prosecuting Attorney's office. After reviewing all of the available information, the offender's name will be released unless there is a reason not to release it.
7. If the FSC cannot reach consensus, the decision to release the name to the investigating agency will be made by the Attorney General or his/her designee.

2.7.4 Partial Match Procedure

2.7.4.1 Initiating the Partial Match Evaluation

The CODIS State Administrator will verify that the candidate partial match meets the defining criteria in the FBI CODIS Bulletin BT0726006. When a partial match occurs that has at least 15 shared STR alleles with an offender, the CODIS State Administrator will contact the local laboratory's CODIS administrator to confirm that the case is not yet solved. If the case is still active, the case investigator should be notified of the partial match by the local CODIS laboratory and a request for partial match follow-up may be made by the investigating agency.

Partial matches that occurred prior to the date of this procedure will be addressed on a case-by-case basis by the CODIS State Administrator.

Additionally, the investigating law enforcement agency may request an evaluation of their forensic profile to determine if any partial matches exist or are generated when searched.

All requests to initiate a partial match evaluation will be directed to the Familial Search Committee (FSC).

2.7.4.2 Expected Match Ratio (EMR) and Expected Kinship Ratio (EKR) Evaluations

The EMR and EKR values shall be calculated using the Partial Match Calculator as detailed in *SWGDAM Recommendations to the FBI Director on the "Interim Plan for the Release of Information in the Event of a 'Partial Match' at NDIS".* The EMR and EKR values may be used by the FSC in determining further evaluations of partial matches. The partial match MAY be useful if either the EMR or EKR satisifes the following thresholds:

- At least one of the database values is greater than or equal to 1.0 and
- the other values are greater than or equal to 0.1

The FSC may terminate further evaluation of the partial match based upon the EMR and EKR values.

2.7.4.3 Partial Match Request

Prior to initiating this procedure under any circumstances listed above, a written request will be submitted by the chief law enforcement officer of the investigating agency to the Director of the Michigan State Police Forensic Science Division.

Among the essential criteria these critical items require compliance prior to initiation of this procedure:

- The requesting agency will have successfully determined the Y-STR haplotype of the forensic unknown using amplification chemistry approved by the MSP. If the original case was tested by MSP, the Y-STR analysis will be completed by MSP.
- The requesting agency will have considered, attempted, or succeeded in obtaining single source DNA profile at all of the CODIS core 13 loci of the forensic unknown with an STR amplification kit approved by MSP.

2.7.4.4 Preliminary Standard Search

Upon receipt of direction and authorization from the FSC, the CODIS Administrator will receive the CODIS Match ID.

- To confirm that a direct match to a perpetrator is not present, a manual, standard moderate stringency search will be conducted of the forensic unknown against the offender and forensic indexes in the database immediately prior to initiating any partial match processing. The resulting state match detail report(s) will be retained in the file.
- In the event of an offender hit, standard confirmatory processing will be initiated, the partial match process will be terminated and the FSD immediately notified.
- If a new, additional "partial match" is obtained, it will be noted and the State Match Detail Report will be retained. The specimen involved will, at the discretion of the FSC, be considered for inclusion in the Y-STR processing list that is generated later in the process.

2.7.4.5 12 High + 1 Mismatch Search

As an additional quality assurance step, a search of the forensic unknown index will be conducted at high stringency at twelve loci with one mismatch allowed. Any resultant candidate matches under these search conditions will be documented and evaluated as potential offender hits in consultation with the FSC.

In the event of an offender hit, standard confirmatory processes will be initiated. The partial match process will be terminated and the FSC immediately notified.

2.7.4.6 Y-STR Typing

Y-STR typing of the partially matched offender sample will be performed by the MSP using validated Y-STR methods.

The Y-STR haplotype of the partially matched forensic unknown is compared against the set of Y-STR types obtained from the partially matched offender sample. The presence or absence of concordant Y-STR types is evaluated using standard interpretation guidelines and documented in the case file.

2.7.4.7 Reporting Results

The FSD Biology Program Coordinator will notify the FSC of the existence or absence of offender Y-STR types concordant with the Y-STR haplotype of the target forensic unknown.

Where no appropriate investigative leads are identified, the FSC will draft a letter communicating the negative result of the partial match evaluation to the requesting agency.

Where a potential investigative lead exists, the MSP will initiate a background investigation and the handling of the partial match will be conducted in the same manner as a candidate from a familial search.

2.8 DNA Technical Leader Vacancy

2.8 DNA Technical Leader Vacancy

The following procedure shall be followed in the event that the DNA Technical Leader (Biology Program Coordinator) position is vacated.

2.8.1

A current Michigan State Police employee that meets the experience and educational qualifications as outlined in the FBI's Quality Assurance Standards for DNA Testing Laboratories and DNA Database Laboratories shall be immediately appointed on an interim basis.

2.8.2

Upon appointment of the interim DNA Technical Leader, the State CODIS Administrator shall be notified. The State CODIS Administrator shall then notify the NDIS Custodian.

2.8.3

A permanent selection of a DNA Technical Leader shall be completed through Departmental procedures within one year of the vacancy.

2.8.4

If there is not a qualified individual to appoint as interim DNA Technical Leader, all DNA casework and CODIS operations shall be halted.

2.8.5

When a qualified interim DNA Technical Leader is not immediately available, Appendix B of the Quality Assurance Standards for Forensic DNA Testing Laboratories and DNA Database Laboratories shall be completed and forwarded to the NDIS Custodian within 14 days of the vacancy with a contingency plan for filling the DNA Technical Leader position.

2.8.6

DNA casework and CODIS operations cannot be re-initiated until the contingency plan has been approved by the FBI.

2.8.7

A documented review of the following must be completed by the interim and/or permanent DNA Technical Leader:

2.8.7.1

Validation studies and methodologies currently in place.

2.8.7.2

Educational qualifications and training records for currently qualified scientists.

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MICHIGAN STATE POLICE FORENSIC SCIENCE DIVISION

2.10 Interpretational Guidelines for AB 3500/3500xL STR Profiles-PowerPlex® Fusion

2.10 Interpretation Guidelines for AB 3500/3500xL STR Profiles-PowerPlex® Fusion

2.10.1 Step 1: INTRODUCTION

Short Tandem Repeats, or STRs, are genetic markers that contain short repeated sequences of DNA base pairs. These repeated sequences are typically in the 3-7 base pair size range. They are distributed throughout the human genome and represent a good source to differentiate individuals. For this reason, STRs are said to be highly polymorphic. STRs may be detected through the use of a combination of techniques including the Polymerase Chain Reaction (PCR) and capillary electrophoresis. The PCR process amplifies a specific location (locus) on the chromosome utilizing fluorescently labeled primers and the enzyme *Taq* Polymerase. Capillary electrophoresis separates the DNA fragments by size, allowing for allele designations. The allele designation is based upon the number of repeat sequences in the DNA fragment. Autosomal STR genotypes represent DNA types from both maternal and paternal origins.

The PowerPlex® Fusion chemistry kit allows for the amplification of 22 autosomal STR genetic markers plus the Amelogenin (gender) locus and a Y-chromosome STR. The autosomal STR genetic markers include D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA and D22S1045. The Y-chromosome STR is DYS391. The PowerPlex® Fusion kit utilizes a five color detection system. Fluorescein (FL) is utilized for Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317 and Penta E. The dye set JOE is utilized for D16S539, D18S51, D2S1338, CSF1PO and Penta D. TMR-ET is utilized for TH01, vWA, D21S11, D7S820, D5S818, TPOX and DYS391. The dye set CXR-ET is utilized for D8S1179, D12S391, D19S433, FGA and D22S1045. All of the loci are co-amplified in a single reaction, or tube, and separated by a single electrokinetic injection on an ABI 3500/3500xL electrophoresis genetic analyzer utilizing Promega's CC5 Internal Lane Standard 500 sizing standard. Data analysis can be accomplished using ABI's GeneMapper® ID-X v. 1.4 software in combination with Promega's PowerPlex® Fusion bins and panels set.

A summary of each locus within the PowerPlex® Fusion amplification kit and the designation are displayed below:

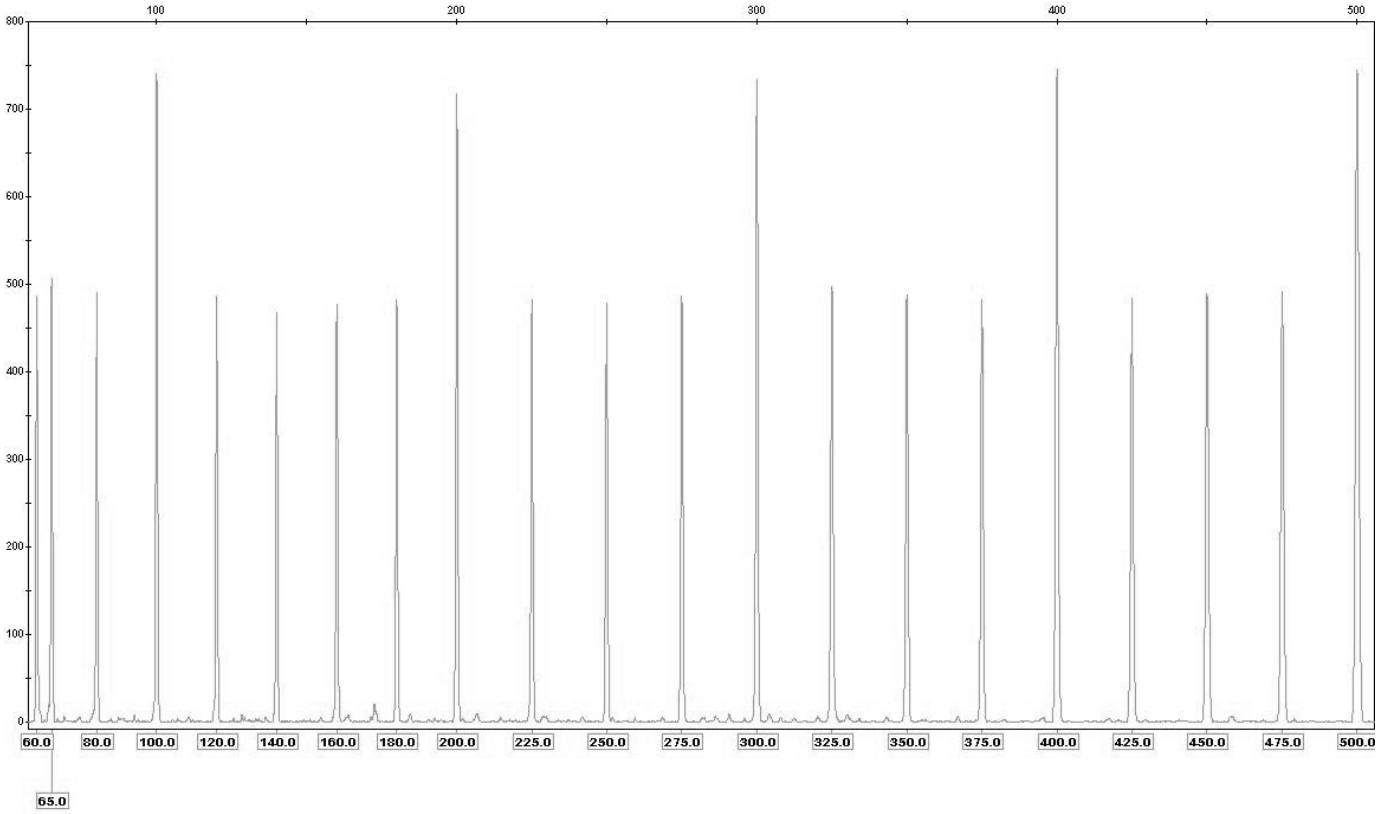
Genetic Marker	Min. Size (bp)	Max. Size (bp)	Repeat Structure	Ladder Alleles
Amelogenin	89	95	N/A	X,Y
D3S1358	103	147	4	9-20

D1S1656	161	208	4	9-14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D2S441	214	250	4	8-11, 11.3, 12-17
D10S1248	256	280	4	8-19
D13S317	302	350	4	5-17
Penta E	371	466	5	5-24
D16S539	84	132	4	4-16
D18S51	134	214	4	7-10, 10.2, 11-13, 13.2, 14-27
D2S1338	224	296	4	10, 12, 14-28
CSF1PO	318	362	4	5-16
Penta D	377	450	5	2.2, 3.2, 5-17
TH01	72	115	4	3-9, 9.3, 10-11, 13.3
vWA	127	183	4	10-24
D21S11	203	259	4	24, 24.2, 25, 25.2, 26-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2 36-38
D7S820	269	313	4	5-16
D5S818	321	369	4	6-18
TPOX	393	441	4	4-16
DYS391	442	486	4	5-16
D8S1179	76	124	4	7-19
D12S391	133	185	4	14-17, 17.3, 18, 18.3, 19-27
D19S433	193	245	4	5.2, 6.2, 8-12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2
FGA	265	411	4	14-18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26-30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
D22S1045	425	464	3	7-20

2.10.2 Step 2: EVALUATION OF INTERNAL SIZE STANDARDS, ALLELIC LADDERS AND CONTROLS

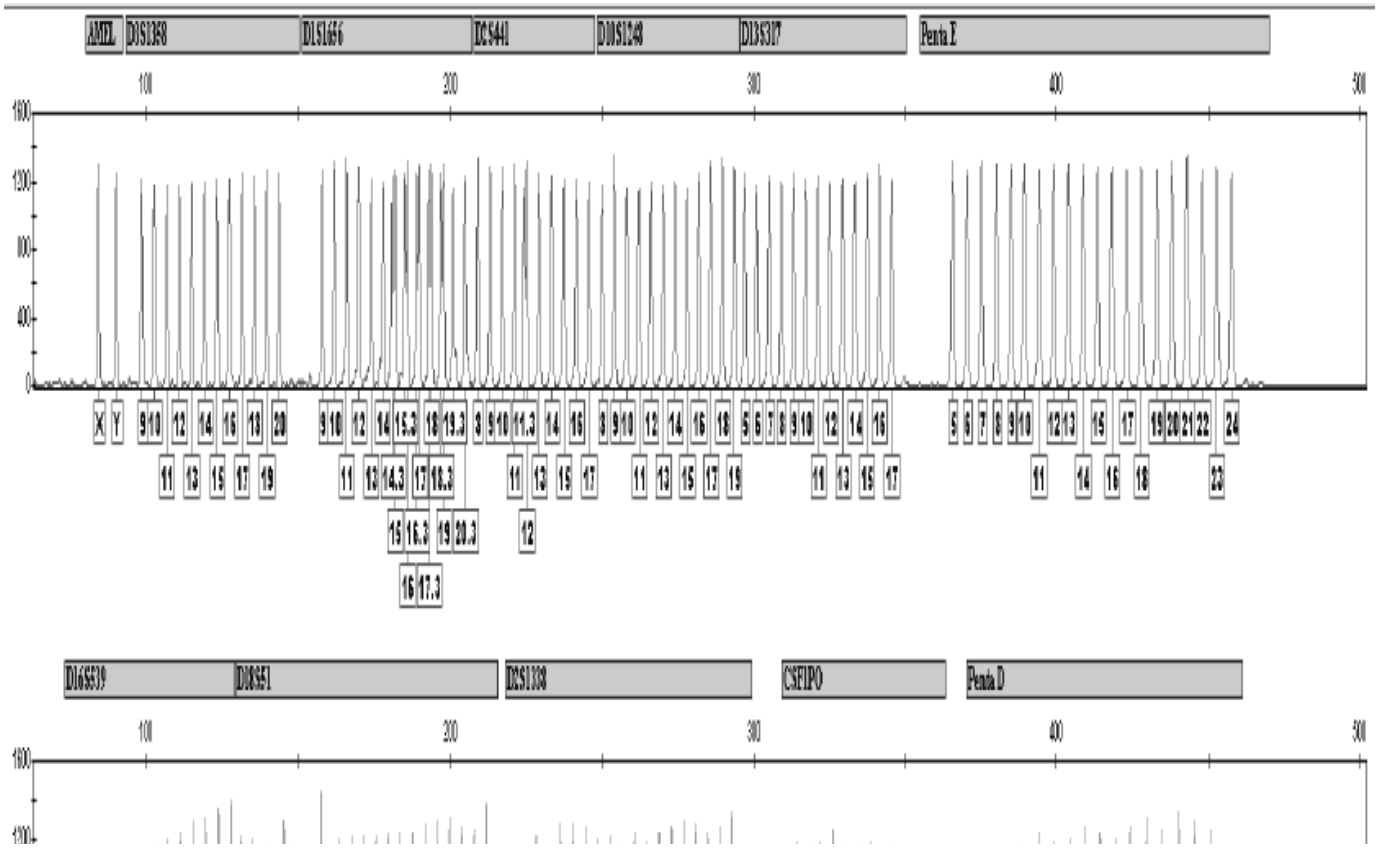
2.10.2.1 Internal Size Standard

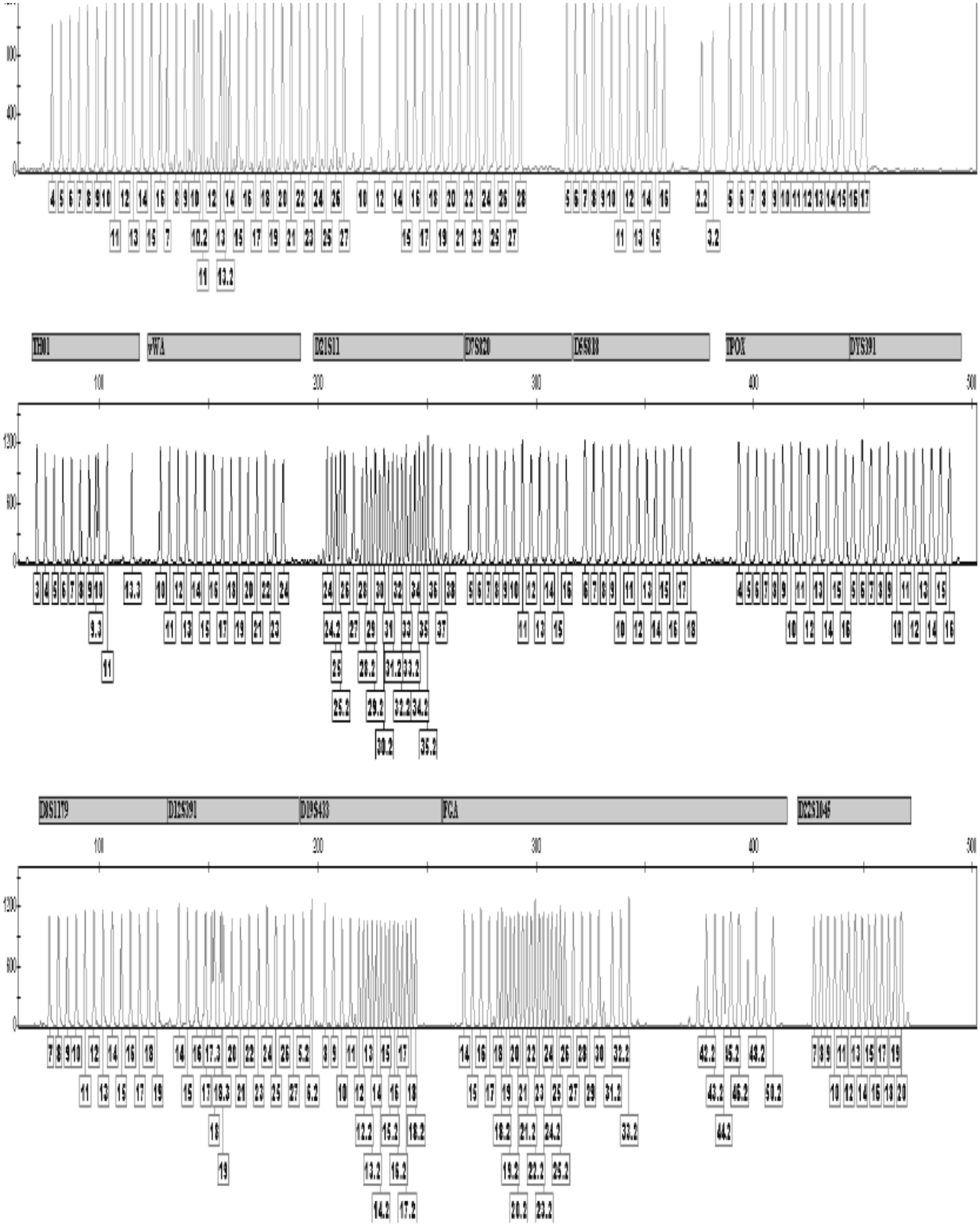
The Internal Lane Standard (ILS 500) shall be examined to verify that the fragments have been properly labeled as depicted below. All size standard peaks should be greater than or equal to the analytical threshold of 75 RFUs. ILS 500 peaks that do not exceed the 75 RFUs analytical threshold should be evaluated with caution, ensuring that the low RFUs are not indicative of the overal performance of the detection and amplification.



2.25.2.2 Allelic Ladder

The Allelic Ladder shall be examined to verify that the types have been properly assigned as depicted in the allelic ladder below and that all alleles are greater than or equal to 250 RFUs.





2.10.2.3 Positive Controls

Positive Amplification Control (2800M)

Purpose: To provide a documented positive human DNA control to monitor the amplification process. The Positive Amplification Control is run with each amplification set and kit.

The Positive Amplification Control profile is:

Genetic Locus	2800M Genotype
Amelogenin	X,Y
D3S1358	17,18
D1S1656	12,13
D2S441	10,14
D10S1248	13,15
D13S317	9,11
Penta E	7,14
D16S539	9,13
D18S51	16,18
D2S1338	22,25
CSF1PO	12,12
Penta D	12,13
TH01	6,9.3
vWA	16,19
D21S11	29,31.2
D7S820	8,11
D5S818	12,12
TPOX	11,11
DYS391	10
D8S1179	14,15
D12S391	18,23
D19S433	13,14
FGA	20,23
D22S1045	16,16

Internal Laboratory Control (ILC): NIST-Traceable Standard

Purpose: The Internal Laboratory Control originated as an unknown male whole blood sample preserved in K_3-EDTA. Bloodstains were prepared from the whole blood on filter paper. The Internal Laboratory Control was analyzed against the NIST SRM 2391C, making it a NIST-traceable standard.

The Internal Laboratory Control is intended to provide a documented positive human DNA control to monitor the complete DNA analytical process. The Internal Laboratory Control is run with each case, or batch of cases, and is processed with the known samples (reference DNA samples). An Internal Laboratory Control sample shall be included for each stain extraction method utilized (Tissue/Hair or Stain Extraction Buffer) for a case or batch of cases. The Internal Laboratory Control is amplified with each amplification kit and the same thermal cycler model as the samples it is controlling. Additionally, the Internal Laboratory Control is run on each Genetic Analyzer model utilized in each case, or batch of cases it controls. It is acceptable to extract the Internal Laboratory Control with the evidentiary samples only for instances that a batch and/or case do not contain known samples or the known samples are extracted with a different extraction procedure with their own Internal Laboratory Control sample. An Internal Laboratory Control does not need to be included in batches that only contain differential extraction types.

The Internal Laboratory Control (ILC) profile is:

Genetic Locus	ILC Genotype

Amelogenin	X,Y
D3S1358	14,16
D1S1656	14,16
D2S441	13,15
D10S1248	15,17
D13S317	11,12
Penta E	8,16
D16S539	11,11
D18S51	12,21
D2S1338	19,20
CSF1PO	8,10
Penta D	2.2, 2.2
TH01	7,7
vWA	13,16
D21S11	30,33.2
D7S820	10,12
D5S818	11,11
TPOX	11,11
DYS391	11
D8S1179	13,14
D12S391	18,23
D19S433	14.2,15
FGA	24,24
D22S1045	14,15

Positive Control Performance Criteria - (2800M and Internal Laboratory Control)
Genotypes must match the expected genotype at each locus. The relative fluorescent intensity must meet the minimum analytical threshold of 250 RFUs. Exceptions to meeting the minimum analytical threshold of 250 RFUs are outlined below. Because the 2800M and Internal Laboratory Control samples are single source in nature, it is acceptable to have homozygous alleles below the 900 RFUs Stochastic Threshold. It is acceptable for the 2800M and Internal Laboratory Control samples to have Peak Height Ratios (PHRs) less than the expected 50% for heterozygous markers. The 2800M and Internal Laboratory Control controls are single source samples. Alleles with very high RFUs that exhibit potential oversaturation of the genetic analyzer detector as represented by flat peak tops and/or associated elevated stutter peaks may be interpreted if the peaks are symmetrical, sharp and potential artifacts do not interfere with the interpretation of the electropherogram. Alleles that do exhibit oversaturation as described previously must be reanalyzed by reamplification and/or reinjection at reduced injection conditions.

Performance failures of Positive Controls

Incorrect genotypes - If any positive control (2800M or Internal Laboratory Control) has genotypes that are not the expected genotypes at all genetic markers, all samples within the batch must be re-amplified with the same kit initially used. A different lot of the amplification kit may be used if the initial lot is no longer available. Results from samples that do not have sufficient material to re-amplify are invalid and shall not be interpreted or reported.

If any positive control (2800M or Internal Laboratory Control) has alleles at some or all loci that do not meet the 250 RFUs analytical threshold, the entire amplification set shall be evaluated to see if the trend is observed across all samples within the batch. If there is a trend that the entire run is showing characteristics exhibited in the positive control, the amplification is voided and the set shall be re-amplified. If the second positive control associated with the amplification run (Internal Laboratory Control or 2800M) meets expected standards and the over-all characteristics of the run do not mirror the performance of the failed control (RFUs values lower than anticipated), the data can be reported.

Instances where the positive control performance did not meet acceptable standards (greater than or equal to 250 RFUs and/or expected genotype), a statement in the report shall be included indicating the positive control performance failure. Instances where additional action is taken to achieve acceptable positive control performance (increased injection time, re-amplification of the ILC etc...), may be reported without a

statement in the report.

2.10.2.4 Negative Controls

Purpose: To provide a documented control to monitor reagents, test environment and processing procedures associated with the complete PCR analytical process. All negative controls shall correlate with the most restrictive samples in the DNA batch such as extract recovery volume, amplification volume and electrophoresis injection times. All negative controls shall be amplified on the same thermal cycler model and analyzed on the same genetic analyzer model as the samples they are controlling. All extraction sets where potential additional testing using chemistries or testing conditions necessitate additional amplifications shall include two reagent controls for each extraction type. The replicate with the highest DNA concentration for the amplification chemistry being utilized (autosomal-STR or Y-STR) shall by continued through STR typing. The replicate with the lowest DNA concentration shall be archived for possible future additional testing. If both replicates of the reagent control result in an undetected DNA concentration, one reagent control is archived and the other is carried forward to STR amplification.

An EXTRACTION SET is defined as a set of samples and/or controls that are extracted concurrently, using the same extraction procedure and reagent lot numbers. Evidentiary and reference DNA sample sets requiring separation by time and/or space shall have their own extraction reagent blank(s).

STAIN REAGENT CONTROLS - A blank control that is processed with the stain extraction, Chelex extraction or Promega's Tissue and Hair Extraction Kit procedure.

The Stain Reagent Control is set up with each of the stain extraction sets.

DIFFERENTIAL REAGENT CONTROL - A blank control that is processed with the differential extraction procedure.

The Differential Reagent Control is set up with each differential extraction set.

AMPLIFICATION NEGATIVE CONTROL - A blank control that is processed with the amplification procedure.

The Amplification Negative Control is set up with each set of amplifications run concurrently on the same thermal cycler. The Amplification Negative Control should be comprised of the same solution (water or TE $^{-4}$) that is used in the normalization of DNA samples within the amplification set.

Negative Controls - Performance Criteria

Alleles should not be observed in these samples above the 250 RFUs reporting threshold (analytical/detection).

Peaks greater than 75 RFUs and corresponding to allele positions should be evaluated carefully. If it is determined that peaks correspond to alleles and a potential genotype proceed to the Failure of Negative Controls section, below.

Failure of Negative Controls

Failure of negative controls can result from a variety of situations. Each failure will be evaluated on a case by case basis.

The point and source of the failure should be determined, when possible.

If the alleles and/or genotypes observed in the negative control(s) for a set of reactions are the same as the genotype that supports an inclusion, the data for those related samples is voided and may require re-extraction of the original item of evidence.

If the alleles or genotype observed are not associated with a genotype that supports an inclusion, the data may be interpreted cautiously. If the results are reported, the failed control must be reported in the written report.

2.10.2.5 Control Failures

Performance failure in the Positive and/or Negative controls may require additional actions to be taken. Instances of performance failure shall be reported to the Unit Supervisor and/or DNA Technical Leader. For cases of drop-in events, the unit supervisor and technical leader have the ability to instruct an analyst move forward using report wording regarding controls under 2.10.10.5. Refer to the Quality Manual section for Discrepancies and Corrective Actions.

2.10.3 Step 3: ALLELE DECLARATION

2.10.3.1 Allele Declaration

Alleles are declared when they meet the following criteria

The allele is within a ± 0.5-bp "window" around the size obtained for the corresponding allele in the Allelic Ladder.

The peak height is 250 RFUs (Relative Fluorescent Units). The 3500/3500xL Genetic Analyzer Analytical Threshold (AT) is 250 RFUs. The analytical threshold is defined as the minimum peak height in RFUs for assigning an STR type to an observed PCR peak. It is set based upon instrument sensitivity, baseline noise and amplification chemistry . Peaks observed within electropherograms that do not meet or exceed the analytical threshold of 250 RFUs are not labeled by the 3500/3500xL Genetic Analyzer using GeneMapper® ID-X v. 1.4 software and are not interpreted.

Stutter peaks are a common artifact associated with the amplification of DNA using the Polymerase Chain Reaction and are due to strand

slippage of the enzyme Taq Polymerase. Stutter peaks are often observed one repeat unit less than the actual allelic peak (N-3, N-4, N-5), and to a lesser extent one repeat larger than the actual allelic peak (N+3, N+4, N+5). Within a genetic locus, larger alleles may give stutter peaks that are larger than smaller alleles. Note: The major peak must be not have indications of over-saturation of the genetic analyzer detector. If the main peak is over-saturated in a reference sample and an allele is declared in a stutter position, the sample must be re-run or re-amplified to obtain a main peak that does not show indications of over-saturation.

If the peak height ratio is greater than or equal to the stutter threshold the allele is declared. It should be noted that a perceived N-3, N-4 or N-5 stutter peak may slightly exceed the established stutter threshold dependent upon a number of factors such as stochasm. One of the instances whereby the stutter percentage may exceed the established threshold occurs when a heterozygous pair of alleles are separated by 2 repeat units. The presence of a negative stutter peak for the larger allele, combined with a positive stutter peak from the smaller allele, may cause the artifact to exceed the established threshold. This observation should be evaluated cautiously, taking the full profile and the presence of additional minor contributors into consideration.

If the peak height ratio is less than the stutter threshold, the allele is not declared.

Genetic Locus	Negative Stutter (one repeat)
D3S1358	≤ 11.9%
D1S1656	≤ 14.2%
D2S441	≤ 9.2%
D10S1248	≤ 12.4%
D13S317	≤ 9.8%
Penta E	≤ 7.6%
D16S539	≤ 10.2%
D18S51	≤ 14.6%
D2S1338	≤ 13.9%
CSF1PO	≤ 9.5%
Penta D	≤ 6.8%
TH01	≤ 4.6%
vWA	≤ 11.2%
D21S11	≤ 11.6%
D7S820	≤ 11%
D5S818	≤ 9.5%
TPOX	≤ 5.5%
DYS391	≤ 8.7%
D8S1179	≤ 10.9%
D12S391	≤ 15.8%
D19S433	≤ 11%
FGA	≤ 12.1%
D22S1045	≤ 16.4%

Amelogenin is not an STR genetic marker, therefore, it does not have the same tendency to produce a stutter peak. Genetic locus D22S1045 tends to produce positive stutter at a higher level than other markers within the Fusion kit and has a positive stutter threshold of 8.6%. Additionally, genetic locus D1S1656 has an N-2 stutter threshold set at 3.6%.

2.10.3.2 Variant - (True Off-ladder Alleles)

Off-Ladder Alleles-A tetra-nucleotide repeat STR will have allele names that differ by four base pairs and penta-nucleotide repeats will vary by five base pairs, and so on. Microvariants differ from common alleles by one or more base pairs (less than the consensus repeat unit). Because microvariants and some rare alleles often do not label the same as consensus alleles present in the reference allelic ladder, they are often referred to as "off-ladder" alleles. GeneMapper® ID-X v. 1.4 incorporates into the bin set many of the common microvariants observed. If an off-ladder allele within the reference allelic ladder locus range is not recognized by GeneMapper® ID-X v. 1.4, it can be assigned an allele name

by calculating the number of base pairs separating it from the nearest lower allele in the reference allelic ladder. The number of base pairs is added to the allele call of the nearest lower allele separated by a decimal. For instance, an allele at 257.51 base pairs is labeled in GeneMapper® ID-X v. 1.4 as an off-ladder allele. The next nearest lower allele is a 28 at 256.64 base pairs in size. The difference is near 1 base pair. The allele is designated 28.1. The migration differences in the sample as compared to the reference allelic ladder may be considered in determining the base pair size difference. If an off-ladder allele is designated within GeneMapper® ID-X v. 1.4 and it is outside of the allelic ladder locus range, the allele call shall be designated as > the largest allele in that allelic ladder locus or < the smallest allele in the allelic ladder for that locus, where applicable. Reference the appropriate section of this document regarding any requirements for re-amplification of "off-ladder" alleles.

Alleles designated "OL" by the GeneMapper® ID-X v. 1.4 software and falling above the largest or below the smallest designated allele defined by GeneMapper® ID-X v. 1.4, will be designated as either greater than (>) or less than (<) the respective real ladder allele.

Virtual alleles falling above the largest or below the smallest "real" peak of the allelic ladder may not be recognized for CODIS entries. See Biology Procedure 2.17 for a listing of alleles eligible to be entered into CODIS.

Alleles designated "OL" should be evaluated based on the base pair size and recorded as previously noted. Any subsequent comparison of these "OL" alleles will also be based on the base pair size.

The minimum allele frequency for the locus will be used in the statistical estimation calculation.

2.10.3.3 Confirmation of off-ladder alleles and tri-allelic patterns

Off-ladder alleles and tri-allelic patterns observed in samples used for current or future inclusionary purposes or CODIS entry (off-ladder alleles only) may be confirmed by re-amplification when sample is available. In the case of multiple samples exhibiting the off-ladder allele or tri-allelic pattern amplified on the same thermal cycler and at the same time, only one sample of the amplification set may be re-amplified. In the case of multiple samples exhibiting the off-ladder allele or tri-allelic pattern amplified on separate occasions, re-amplification is not required.

Off-ladder alleles observed in reference samples, where the reference sample is excluded as a potential donor based on multiple loci or evidentiary profiles were not observed on the case, may not require confirmation.

2.10.4 Step 4: ARTIFACT DECLARATION

Artifacts and anomalies are encountered during the fluorescent analysis of Short Tandem Repeats (STR). Many times these artifacts and anomalies exhibit characteristics that are predictable and defined. In these instances it may not be necessary to re-run the sample. Listed below are recommendations for handling these events:

2.10.4.1 General Considerations

The position of the artifact or anomaly in relation to the allelic ladder should be considered in each instance.

Both the Raw Data and GeneMapper® ID-X v. 1.4 data should be evaluated to make the interpretation of the artifact or anomaly.

The characteristics of the artifact or anomaly should be carefully evaluated before changes in injection time, dilution adjustment or re-amplification is initiated.

2.10.4.2 Events Associated with Amplification

Incomplete "A" nucleotide addition

An allele may display a leading peak shoulder approximately one base pair shorter than the allele with which it is associated. This is a result of incomplete adenine addition during the final extension cycle. This phenomenon can be associated with increased target DNA or the presence of *Taq* Polymerase inhibitors.

Alleles exhibiting minor shoulders in which the peak base is narrow and the major peak is sharp and well defined may be called without further processing.

If the shoulder peak interferes with the interpretation and/or exhibits major shoulders and broad peak bases, the sample should be re-amplified at a lower concentration or further manipulated to reduce/eliminate the effects of inhibition.

Notation

The incomplete "A" nucleotide addition should be documented with the base pair sizes and noted on the electropherogram as "-A".

Amplification inhibition

Amplification of genetic markers utilizing the Polymerase Chain Reaction (PCR) is an enzymatic reaction. Certain conditions and/or substances may inhibit the reaction. The results of inhibition may generally be noted at two steps during the analytical process, qPCR DNA quantitation and STR detection.

DNA samples exhibiting inhibition at the quantitation stage may show an increase in Ct values and/or lack of IPC amplification. Samples exhibiting inhibition should be evaluated cautiously. Dependent upon sample type, quantity/volume and qPCR data, the sample may be re-extracted, diluted or re-phenol-ed to remove the inhibitor prior to re-quantification and/or amplification for STRs.

DNA samples exhibiting inhibition at the STR detection stage typically show an inhibition "pattern" that may include an overall reduction in allelic

RFUs and/or a lack of sensitivity at genetic loci with larger base pair sizes. Samples exhibiting inhibition effects for STR amplification with PowerPlex® Fusion typically show a reduced RFU level for the Amelogenin marker. DNA samples exhibiting inhibition at the STR detection stage may be re-extracted, diluted or re-phenol-ed to remove the inhibitor prior to re-amplification to remove the effects of the inhibitor. Dependent upon the option selected, the sample(s) may need to be re-quantified.

2.10.4.3 Events Associated with Capillary Electrophoresis

Elevated Stutter Peaks

Single Source Samples (Known)-

Stutter outside the defined range of stutter for a single source sample should be evaluated with respect to the entire profile. If it is judged that the sample profile is characterized by good balance at all of the loci the sample does not need to be rerun.

If the sample does not show good balance then the sample should be re-run or re-amplified.

Notation: Note the stutter percentage on the electropherogram.

Forensic Unknowns

Samples in which the overall profile is consistent with a single source origin, but a peak(s) positioned at the stutter position (n+4, n-4 or n-5 dependent upon the locus repeat motif) exceeds the stutter criteria and reporting threshold, the sample may be re-amplified. If the peak(s) is reproducible upon re-amplification, or if the sample is not re-amplified, then the reported conclusion should indicate that the n+4, n-4 or n-5 peak could be attributed to either a triallelic pattern, possible additional donor(s) or an artifact (elevated stutter).

Samples in which the overall profile is consistent with a mixed sample, elevated stutter should not be considered.

Notation

Note the stutter percentage on the electropherogram.

Fluorescent Pull-Up

Fluorescent pull-up occurs when a peak on an electropherogram, represented by a DNA type, has a sufficiently high RFU value that the matrix/spectral is unable to compensate for the overlapping color fluorescence. This produces a peak in a subsequent color(s) with the same, or nearly the same, base pair size as the peak represented by a true DNA type.

Fluorescent pull-up must be documented on the electropherogram with the base pair sizes of the peaks involved and a notation indicating pull-up.

Electropherograms that contain a possible pull-up peak labeled by GMID-X v. 1.4 as an OL allele may be re-injected or re-amplified to remove the artifact or labeled as a "pull-up" peak. Electropherograms that are indicative of a single source profile that contain a peak deemed as a possible pull-up peak but is labeled by GMID-X v. 1.4 as an allele may be re-injected or re-amplified to remove the artiact or labeled as a "pull-up" peak. Electropherograms that are indicative of a mixed donor profile that contain a peak deemed as a possible pull-up but is labeled by GMID-X v. 1.4 as an allele shall be re-injected or re-amplified to remove the artifact.

Note "pull-up" on the electropherogram.

Fluorescent Spiking

A fluorescent spike occurs due to the presence of a particle and/or bubble within the capillary. A peak(s) is generated on the electropherogram due to the generation of fluorescence from the laser as it interacts with capillary bubbles and/or particles.

If the spike is observed in the same position in two or more dyes in the electropherogram, the sample does not have to be rerun. For instances whereby the fluorescent spike falls between bins and cannot be added to the peak label within GeneMapper ID-X v. 1.4, the base pair sizes can be determined by placing the cursor over the peak. The base pair size shall be noted in the case file as supporting documentation.

A fluorescent spike may be observed in a single color in very rare instances. Typically the peak morphology is not consistent with peaks represented by DNA alleles. A single color fluorescent spike that is >250 RFUs shall be re-injected to distinguish between low intensity alleles and fluorescent artifacts. If the artifact is removed, the interpretation may proceed. If the artifact is reproduced the peak should be re-evaluated as a potential DNA allele.

Notation

Note on the electropherogram or within the case file "fluorescent spike" or "f-spike", with base pair size noted.

2.10.4.4 Peaks that exhibit over-saturation

Single source samples (Known)

If the sample exhibits good balance and well-formed peaks the sample does not need to be rerun. Samples with detector oversaturation shall be re-injected or re-amplified to achieve lower RFUs.

If the sample exhibits exaggerated stutter peaks, exaggerated minus "A" and flat-lined or inverted peaks the sample must be re-injected or re-amplified.

Notation

Note on the electropherogram "possible over-saturation".

Forensic Unknown

The sample should be rerun to bring the peak height down to an RFU level that does not exhibit oversaturation. If the sample rerun is accompanied with unbalanced conditions, the sample should be re-amplified. If an allele is unable to be reduced below saturation, the locus should be reported as inconclusive.

Loci reported as inconclusive shall include a notation of "inc" on the electropherogram.

Amelogenin peaks exceeding saturation in known reference and evidentiary samples don't need to be re-injected at lower injection times and no notation is necessary.

2.10.4.5 Additional Artifacts

Additional artifacts may be recognized during interpretation such as alleles associated with non-human sources, raised baseline or non-allelic peaks associated with fluorescent dyes. These artifacts should be interpreted with caution and supporting documentation (manufacturer's communications, literature etc...) included within the case file.

2.10.5 Step 5: GENERAL SAMPLE CONSIDERATIONS FOR INTERPRETATION

2.10.5.1 Batch Comparisons

A comparison of DNA profiles within a batch of cases must be completed to determine the potential for contamination. Comparisons may be limited to DNA profiles/types that have probative value. Documentation of completion of this procedure must be included within the case file. Observations of potential contamination shall be reported to the Unit Supervisor and/or DNA Technical Leader.

2.10.5.2

All available loci and alleles ≥ the 250 RFU analytical threshold should be utilized during STR interpretation. It should be noted that peaks not represented by an allele (artifacts) do not need to be included within the interpretation.

Data generated from the same extract and amplification may be interpreted across no more than two electropherograms if the profiles obtained are consistent with one another. However, any given locus shall only be interpreted from a single electropherogram.

2.10.5.3 Stochastic Effects

Stochastic effects may be observed when very low levels of DNA are amplified, resulting in low RFUs peak heights on electropherograms. Stochastic effects are observed in resulting peak height ratios of heterozygous peak pairs, allelic dropout, locus dropout and increased stutter above the established locus stutter threshold. A Stochastic Threshold may be used to alert the DNA scientist to the possibility that all of the DNA typing information may not have been detected for a given sample. The Stochastic Threshold is specific to the quantitation method (Plexor® HY and the AB 7500), amplification chemistry and method (PowerPlex® Fusion on the AB 9700 thermal cycler at 30 cycles), capillary electrophoresis instrumentation (3500/3500xL Genetic Analyzers) and other general laboratory procedures. The Stochastic Threshold for PowerPlex® Fusion is set at 900 RFUs. The stochastic threshold may be applied in a number of different scenarios as a factor in determining if undetected alleles may exist. The Stochastic Threshold and its use in DNA profile interpretations will appear in the Single Donor Reference, Single Donor Evidentiary, Distinguishable Mixture, Indistinguishable Mixture and Partial Profile sections.

Peaks representing DNA types that have amplitudes (RFUs) greater than the Analytical Threshold and less than the Stochastic Threshold may exhibit a variety of characteristics. These stochastic characteristics occur at a low frequency and can be generally described as a decrease in the peak height ratios of heterozygous allele pairs, greater potential for allelic or locus dropout, increased stutter as a percentage of the parent DNA type and others.

2.10.6 Step 6: REFERENCE DNA SAMPLE INTERPRETATION

Comparison of reference DNA profiles to evidence DNA profiles shall only be completed when the interpretation of the evidence DNA sample is complete. However, if the evidence sample is considered intimate the expected donor's profile may be used to assist with mixture deconvolution.

A single donor reference DNA sample, in most instances, will result in either homozygous (a single allele) or heterozygous (two alleles) results at each genetic marker tested. In contrast, in most instances the single Y-STR locus DYS391 will only show a single haplotype and the use of a Stochastic Threshold is generally not relevant. Single donor reference DNA samples must meet the Analytical Threshold of 250 RFUs. Homozygous loci, those that have a single allele, must meet the 900 RFUs Stochastic Threshold. In some rare instances, more than two alleles may be represented at a genetic marker of a single donor reference DNA sample. These are generally termed tri-allelic patterns. Tri-allelic markers are not used in statistical calculations, thus are not required to meet the 900 RFUs Stochastic Threshold. If a peak is detected that meets the criteria for pull-up in a reference DNA sample, but is labeled with an allele type (is within an allele bin), consideration must be given to the potential for peak imabalance or a tri-allelic pattern. A pull-up peak that is labeled as an allele may be interpreted as pull-up in a reference DNA sample if it can be reasonably determined that it did not result from a tri-allelic pattern or peak imbalance. It is acceptable to have heterozygous alleles below the 900 RFUs Stochastic Threshold as long as there are no indications of a mixture across the genetic profile. It is

acceptable, for single donor reference DNA samples, to have Peak Height Ratios (PHRs) less than the expected 50% for heterozygous markers greater than the 900 RFUs Stochastic Threshold. However, when Peak Height Ratios are less than the expected level(s), consideration should be given to the potential interpretation implications of the imbalance.

Reference Samples:

A results statement will be provided for each reference DNA sample analyzed prior to conclusion statements for evidentiary samples.

Criteria	Statement
Results at all markers tested	A DNA profile was obtained from ITEM 1
Results at <all markers tested, sufficient for comparison	A partial DNA profile was obtained from ITEM 1 that is sufficient for comparison purposes.
Results at <all markers tested, insufficient for comparison	A partial DNA profile was obtained from ITEM 1 that is not sufficient for comparison purposes.
No profile obtained	A DNA profile was not obtained from ITEM 1

2.10.7 Step 7: SINGLE OR MIXED EVIDENCE DNA SAMPLE?

2.10.7.1 Single Source Data

A single donor evidentiary sample DNA profile should be considered when:

*there are one or two alleles observed at each autosomal genetic locus and a single allele at the Y-STR locus
*heterozygous DNA types appear balanced across loci
*with heterozygous peak height ratios ≥ 50% for PowerPlex® Fusion within a locus
*homozygous loci appear as a single peak exceeding the Stochastic Threshold of 900 RFUs
*stutter peaks are within the pre-accepted locus-specific ranges and

Profiles with heterozygous loci having alleles below the Stochastic Threshold of 900 RFUs may have peak height ratios <50%. Profiles that have heterozygous alleles below the Stochastic Threshold that have peak height ratios < 50% and show no other indications of being a mixed sample may be interpreted as a single source sample with appropriate documentation within the case file and may be utilized in Random Match statistical estimates when comparisons necessitate. For instance, an electropherogram with all homozygous alleles greater than the 900 RFUs Stochastic Threshold, no more than two detected alleles at any autosomal locus, no more than one allele detected at the Y-STR locus and a genetic locus with a possible heterozygous pair having RFUs less than the Stochastic Threshold and a peak height ratio < 50% may be reported as a single source profile if a notation is made within the case file indicating such.

It should be noted that peak height ratios <50% for PowerPlex® Fusion do occur in single donor heterozygotes occasionally, especially when the peak heights are at, or near, the Stochastic Threshold. These samples may be re-amplified to achieve acceptable peak height ratios if sufficient sample remains. If additional sample does not remain, the sample should be addressed on a case-by-case basis and sufficient documentation stored within the case file to support the interpretation.

Rare multi-allele DNA types (3 or more alleles at a single locus) may be observed in single source samples.

Samples considered single source in nature over the entire range of available data, where possible homozygous alleles are observed below the Stochastic Threshold of 900 RFUs, should be interpreted with caution and consideration given to the potential of additional undetected alleles. If a potential homozygous allele with an RFU value below the Stochastic Threshold is going to be interpreted as a homozygote, a notation within the case file shall indicate that the profile is being interpreted as a single source profile and the potential exists for additional undetected alleles. Genetic loci with homozygous alleles below the 900 RFUs Stochastic Threshold shall not be included in Random Match statistical estimates.

2.10.7.2 Mixed Source Data

Evidentiary samples may contain DNA from more than one donor. Multiple donors should be considered when one or more of the following are observed:

*More than two interpretable alleles are observed in at least two autosomal loci or more than one allele at the Y-STR locus. Electropherograms exhibiting more than two alleles at a single autosomal locus or more than one allele at the Y-STR locus may be the result of DNA contributed by more than one donor, a tri-allelic pattern, a duplication, or elevated stutter. The results should be taken in context with the entire sample/case and the interpretation should indicate that the sample results MAY be a mixed DNA sample.

*The presence of a peak in the stutter position that is greater than the stutter peak height ratio threshold.

*Alleles within a genetic locus exhibiting significant imbalance (peak height ratio <50% for PowerPlex® Fusion loci with alleles greater than the Stochastic Threshold of 900 RFUs) must be interpreted in context with the entire profile. Caution should be exercised before declaring a mixture. Possible causes of imbalance at a locus may include degraded DNA, PCR inhibition, low template input DNA, stochastic effects or variations in the primer sequence due to mutation resulting in inefficient amplification of an allele.

2.10.8 Step 8: SINGLE SOURCE EVIDENCE INTERPRETATIONS AND COMPARISONS

2.10.8.1 Determining a match or exclusion

A match between a reference sample DNA profile and an evidentiary single source DNA profile may be declared when the observed alleles at corresponding genetic markers are concordant. An exclusion between a reference sample DNA profile and an evidentiary single source DNA profile may be declared when the observed alleles at corresponding genetic markers are not concordant.

A comparison between two, or more, evidentiary single source DNA profiles where the observed alleles at all genetic markers with interpretable alleles are concordant may be reported as having come from a common donor (i.e. Donor 1/Male 1). Similarly, a comparison between two, or more, evidentiary DNA profiles where the observed alleles at all genetic markers with interpretable alleles are not concordant may be reported as having come from different donors (i.e. Donor 1/Male 1, Donor 2/Male 2 etc...).

Single source evidentiary sample matching a reference sample shall include a Random Match Probability calculation and/or possible source attribution statement.

2.10.8.2 Report Conclusions

Single Source Results Part A:

Criteria	Statement
1-2 alleles at all loci tested	The DNA profile obtained from ITEM 1 is consistent with...
1-2 alleles at all loci tested, 1 additional minor allele at one locus	The DNA profile obtained from ITEM 1 is consistent with...(Part B). One additional DNA type foreign to ITEM 1 was obtained indicating an additional donor(s) or artifact.
1-2 alleles at all loci tested, 1 additional allele at one locus, possible tri-allele	The DNA profile obtained from ITEM 1 is consistent with...(Part B). A possible tri-allelic pattern was observed at locus (LOCUS).
1-2 alleles at <all loci tested	The partial DNA profile obtained from ITEM 1 is consistent with...
1-2 alleles at <all loci tested, insufficient for comparison	A partial DNA profile was obtained from ITEM 1 that is insufficient for comparison purposes.

Single Source Results Part B:

Criteria	Statement
Intimate sample, no victim reference, comparisons necessary	...a female (male) contributor and will be used as the victim reference profile.
Sample on which the victim is expected	...the victim.
Amelogenin X,Y	...a male contributor.
Amelogenin X,X	...a female contributor.
Amelogenin X,X	...an unknown donor.

Single Source Results Part C:

Criteria	Statement
No reference samples submitted for comparison	This profile is suitable for comparison purposes.
Inclusion of a single reference sample	The (partial) DNA profile from ITEM 1 matches the DNA profile from ITEM 2. Include RMP
Inclusion of a reference sample, exclusion of additional reference samples	The (partial) DNA profile from ITEM 1 matches the DNA profile from ITEM 2. ITEM 3 is excluded as a possible donor to ITEM 1. RMP
Exclusion of a reference sample(s)	ITEM 2 is excluded as a possible donor to the (partial) DNA profile from ITEM 1.
Qualitative statement for single source items that are consistent with the victim	ITEM 1 (Victim DNA Reference Sample) is expected to have contributed to ITEM 2 (DNA Evidence Sample).

2.10.9 Step 9: MIXED SOURCE EVIDENCE INTERPRETATIONS AND COMPARISONS

STRMix™ is a fully continuous probabilistic genotyping software application. It may be used by qualified DNA analysts as an interpretation and statistical tool for mixed contributor forensic unknown profiles. STRMix™ is validated to accept most, but not all, 2-, 3- and 4-contributor mixed DNA profiles. Profiles exhibiting significant levels of allelic and/or locus drop-out of one or more of the contributors may not be suitable for analysis using STRMix™. A qualified DNA analyst should be consulted prior to submitting a mixed contributor DNA profile for STRMix™ conside ration. Procedure manual section 2.11 Interpretational Guidelines for AB 3500/3500xL STR Profiles- PowerPlex® Fusion and STRMix™ may be utilized by the qualified DNA analyst at this point by creating an additional DNA record in Forensic Advantage. The case record shall include, at a minimum, the FSD007 Request for Laboratory Exam, electropherogram of the mixed contributor unknown DNA profile and reference DNA profiles. The current Forensic Advantage report may include the following statement for mixed DNA specimens:

Criteria	Statement
Mixed Contributor DNA profile-STRMix™ eligible	The DNA results obtained from ITEM 1 indicate it is a mixture of multiple contributors. Further analysis of this DNA profile will be the subject of a subsequent report.

If STRMix™ is not utilized, this procedure many be continued by a qualified DNA analyst.

2.10.9.1 Mixture Documentation

All samples that result in a mixed-DNA profile where a profile is either deduced for CODIS entry or a probative association has been made shall utilize the DNA Mixture Interpretation Worksheet to assist in the documentation of analyst interpretation. Additional notes and calculations supporting the analyst interpretation shall be noted on the actual electropherogram or in separate paperwork. Additional notes and/or documentation may include peak height ratios, DNA donor ratios, noted artifacts, possible genotypic allele combinations etc... Instances where a mixed DNA profile is obtained that contains a clear and unambiguous major donor with indications of a minor additional donor(s) that are not able to be interpreted do not require the use of the DNA Mixture Interpretation Worksheet.

2.10.9.2 Estimating the number of DNA Contributors

The minimum number of contributors to a mixed DNA sample shall be determined based upon a number of factors. The overall quality of the electropherogram, the locus with the greatest number of interpretable alleles (≥ 250 RFUs), the peak height of alleles within a locus and the presence of possible alleles below the Analytical Threshold should each be considered when determining the number of possible donors to a mixed sample. The observations relied upon to qualify the number of potential donors to a sample shall be documented within the case file. The first step to estimate the number of contributors to a mixed DNA sample is to locate the locus with the greatest number of alleles present. The number of alleles may be dividied by 2. The value may be rounded up to the nearest whole number. For example, a locus may exhibit five alleles resulting from a mixture of 5/2=2.5, or at least three contributors. Second, it may be necessary to evaluate potential allelic activity below the analytical threshold of 250 RFUs to gain insight on the number of potential contributors to a mixed DNA sample. Finally, any potential stochastic issues and allele balance It should be noted that this method only provides an estimate of the minimum number of contributors. Studies indicate it is difficult to determine with certainty the actual number of donors to any given mixture, especially as the number of donors increases.

2.10.9.3 Indistinguishable DNA Mixtures

Indistinguishable mixtures can be defined as mixed DNA samples where the peak height intensities are similar between donors or there is a presence of shared alleles eliminating the possibility of assigning DNA genotypes to potential donors. Indistinguishable mixtures that contain more than two donors shall be interpreted with extreme caution and consideration to the potential for shared alleles resulting in contributions from any given individual below the stochastic threshold. Indistinguishable mixtures that contain more than two individuals with the potential for significant allele sharing and the potential for allele dropout shall be reported as inconclusive and not used for exclusionary purposes.

A DNA evidentiary sample profile determined to be indistinguishable shall be interpreted prior to comparing the results to a DNA reference sample profile. However, if the sample is determined to be an intimate sample, the DNA profile of the known contributor may be used during the mixed profile interpretation. The interpretation shall include a notation of the genetic markers that have DNA alleles that are below the Stochastic Threshold of 900 RFUs. The interpretation shall also include the possible presence of additional DNA alleles that are below the Analytical Threshold. A genetic locus, or genetic loci, that have a DNA type(s) above the Analytical Threshold, but below the Stochastic Threshold, may be included in the development of a conclusion when compared to a DNA reference sample profile.

Exclusions may be reported if it is determined that the set of detectable alleles and/or genotypes in the DNA mixture does not include the alleles and/or genotypes observed from a known reference sample.

Inclusions may be reported for genetic loci that do not have the potential for allelic dropout where the set of alleles exceeding the analytical threshold in the mixture include all of the alleles from the known reference sample. If an individual is included as a potential donor, a statistical calculation for multiple contributors (CPI/CPE method 2.25.8) shall be applied. If the DNA reference sample profile is included in the mixed evidentiary DNA profile at a genetic locus that contains a DNA type(s) below the Stochastic Threshold, that genetic locus shall not be included within the Combined Probability of Inclusion statistical estimate. If all genetic markers for the mixed evidentiary DNA profile have DNA alleles with RFUs below the Stochastic Threshold, the sample shall have a final disposition of uninterpretable due to the complexity of the DNA mixture. Samples that result in an inclusion with very few genetic loci available for use in statistical estimations may be reported either as uninterpretable

or with a Combined Probability of Inclusion statistical estimation. This determination shall be based on analyst discretion and the probative value of the sample. A single statistical estimate, in many instance, should be able to be applied for a mixed DNA sample when comparing multiple reference sample profiles.

Genetic loci where an allele attributed to a known DNA profile is above the Analytical Threshold (allele 1) and an allele is below the Analytical Threshold (undetected) (allele 2) shall be documented in the case file as inconclusive for that locus. If the second allele is not detected, a notation on the electropherogram should indicate possible allelic dropout.

The inclusion or exclusion of an individual from an indistinguishable mixture when the full complement of genetic markers cannot be used shall be determined on a case-by-case basis with considerations to locus base pair size, sensitivity and the potential for allelic dropout.

2.10.9.4 Distinguishable DNA Mixtures

Distinguishable mixtures-Profiles attributed to a major and/or minor donor of a mixed DNA sample may be determined based on a number of factors including differences in signal intensities, allele sharing, the number of contributors and subtracting the contribution of a known contributor's profile. Generally, distinguishable mixtures are only able to be deconvoluted when you have no more than two contributors or you have a clear and unambiguous major donor with multiple minor donors of a three or more person mixture.

Deconvolution of distinguishable mixtures should be completed prior to comparison to known reference samples except in situations when a known contributor's profile is used in the mixture interpretation.

The unknown profile/types can be determined by subtracting the contribution of the known donor from the mixed profile for evidentiary samples. This may be utilized when evaluating DNA profiles from evidentiary samples physically removed from one contributor (i.e. orifice/body swabs, undergarment/clothing and female fractions to assist with interpretation of the male fraction). Documentation of physical removal of undergarments/clothing from the individual shall be included within the case file. If a sample is determined to be intimate in nature and one donor is expected to be within the DNA mixture, the reported conclusion shall include a qualitative statement addressing the assumption. See 2.10.9.6 for standardized wording. In some instances, it may be necessary to determine and/or document sharing of the item with other potential donors.

The approximate ratio of major to minor donors should be determined, when applicable and able to do so. This may be accomplished by comparing peak heights attributed to each donor at genetic locus/loci where the donors are present without overlapping alleles. This ratio may assist with the further interpretation of the mixture.

If a potential allele occurs in a stutter position, the relative input of expected stutter on the overall peak height intensity may be utilized.

Genotypes attributable to each contributor may be assigned where possible. Relative peak height intensities and ratios should be calculated for each donor based upon the mixture ratio for overlapping alleles and stutter where necessary. Pair-wise comparisons of all potential genotypic combinations should be evaluated. A peak height ratio of 50% for genetic loci with alleles greater than the Stochastic Threshold should be maintained to determine major and minor contributors. Alleles that are not balanced within those percentages shall not be used in declaring major and minor contributors.

DNA profiles shall not be attributed, and a subsequent match declared, to a minor donor where more than two reportable donors are observed. Minor donors may either be included or excluded from these mixtures. If a minor donor is included, a CPI/CPE statistical estimate may be applied.

A match between a reference sample DNA profile and a major or minor (2 person mixture only) donor DNA profile may be declared when the attributed alleles are concordant.

A non-match may be declared between a reference sample DNA profile and a major or minor (2 person mixture only) donor DNA profile when the attributed alleles are non-concordant.

It is possible to have genetic markers that are able to be deconvoluted into donor genotypes AND genetic markers that are indistinguishable within an electropherogram. The choice to conduct a Random Match Probability or CPI/CPE statistical estimation for the overall sample electropherogram shall be determined based upon which method gives the most common frequency in the population. If the differences between the Random Match Probability and CPI/CPE statistical estimations are comparable, the estimate including the largest number of genetic markers should be reported and appropriate conclusion utilized. It should be noted that a genetic locus/genetic loci with alleles below the Stochastic Threshold may not be utilized in the CPI/CPE statistical estimate. Both statistical methods may need to be included within the case file to support the choice of which estimate is reported.

2.10.9.5 General Mixture Considerations

Possible mixture combinations at one locus.

Heterozygous + Heterozygous: Four Distinguishable Peaks. No overlapping alleles.

Heterozygous + Heterozygous: Three Distinguishable Peaks. One overlapping allele.

Heterozygous + Heterozygous: Two Distinguishable Peaks. Two overlapping alleles.

Heterozygous + Homozygous: Three Distinguishable Peaks. No overlapping alleles.

Heterozygous + Homozygous: Two Distinguishable Peaks. One overlapping allele.

Homozygous + Homozygous: Two Distinguishable Peaks. No overlapping alleles.

Homozygous + Homozygous: One Distinguishable Peak. Overlapping alleles.

2.10.9.6 Report Conclusions

Intimate samples:

If a sample meets the definition of intimate and the expected individual is included in the DNA results, the following statement must be included in the conclusion statement. If a sample meets the definition of intimate but the expected individual is NOT included in the DNA results, the following statement does not apply.

Criteria	Statement
Meets definition of intimate sample and reference sample utilized for mixture deconvolution	ITEM 1 (DNA Reference Sample) is expected to have contributed to ITEM 2 (DNA Evidence Sample).

Mixture Results Part A:

Criteria	Statement
>2 alleles at ≥1 locus, results at all loci tested	The DNA types obtained from ITEM 1 are consistent with a mixture...
>2 alleles at ≥1 locus, results at <all loci tested	The partial DNA types obtained from ITEM 1 are consistent with a mixture...

Mixture Results Part B:

Criteria	Statement
1-4 alleles per locus, no additional alleles indicated	...of two individuals...
1-4 alleles per locus, may have >4 alleles at a locus or possible additional alleles indicated	...of at least two individuals...
>4 alleles at ≥1 locus	...of three or more individuals...

Mixture Results Part C:

Criteria	Statement
Amelogenin Y present	...including at least one male contributor.
Amelogenin Y not present	...including at least one unknown contributor.
Amelogenin Y present, major male donor	...including a major male contributor.
Amelogenin Y not present, major female donor	...including a major unknown contributor.
Victim included, foreign male present	...including the victim (suspect, elimination or ITEM #) and one male contributor.
Victim included, ≥ 3 foreign alleles at > 1 locus	...including the victim (suspect, elimination or ITEM #) and at least one (male, additional) contributor.
Victim included, foreign donor	...including the victim (suspect, elimination or ITEM #) and one unknown contributor.
Full major male profile, victim as a minor donor	...including a major male contributor and alleles consistent with the victim.
Full major male profile, foreign minor alleles present	...including a major male contributor.

Mixture Results Part D (select multiples as needed):

Criteria	Statement
Major donor deduced, inclusion	ITEM 2 matches the major donor to ITEM 1. (Include RMP)
Major donor deduced, exclusion	ITEM 2 is excluded as a major donor to ITEM 1.
Major donor, no comparisons	The major donor to ITEM 1 is from an unknown donor.
Minor/additional donor deduced, inclusion	ITEM 3 matches the minor/additional donor to ITEM 1. (Include RMP or CPI)

Minor/additional donor deduced, exclusion	ITEM 3 is excluded as a minor/the additional donor to ITEM 1.
Minor/additional donor, no comparison	The minor/additional donor to ITEM 1 is from an unknown donor.
Minor/additional donor, insufficient for comparison	The minor/additional donor to ITEM 1 is insufficient for conclusive association purposes.
Unresolvable mixture, inclusion	ITEM 2 is included as a possible contributor to ITEM 1. (Include CPI)
Unresolvable mixture, exclusion	ITEM 2 is excluded as a possible contributor to ITEM 1.
Unresolvable mixture, no comparison	No comparisons to ITEM 1 were conducted at this time.

Low Level Partial/Low Level Mixture/Heavy Mixture Profile Results:

Criteria	Statement
few loci present, >4 alleles at >1 locus, high probability of a false inclusion	A partial DNA profile of at least three donors was obtained from ITEM 1. Due to the limited data obtained,no conclusions can be made.
few loci present, 1-4 alleles per locus, no additional alleles indicated	A partial DNA profile of two donors was obtained from ITEM 1. Due to the limited data obtained, no conclusions can be made.
few loci present, 1-4 alleles per locus, may have >4 alleles at a locus or possible additional alleles indicated	A partial DNA profile of at least two donors was obtained from ITEM 1. Due to the limited data obtained, no conclusions can be made.
>4 alleles at >1 locus, no major profile, high probability of a false inclusion or all loci tested present, >4 alleles at >1 locus, high probability of a false inclusion or All loci tested present, high probability of allelic dropout high probability of a false inclusion	A mixed DNA profile was obtained from ITEM 1. Due to the complexity of the profile, no conclusions can be made.

2.10.10 Step 10: ADDITIONAL REPORT STATEMENTS

The following Powerplex Fusion autosomal STR report wording guidelines are designed to provide the customer a consistent and accurate report that is understandable by the reader. If a scenario is encountered in DNA casework that is not addressed by these guidelines, report wording shall be provided to the unit supervisor and DNA Technical Leader for consideration and potential inclusion in the procedures.

2.10.10.1 Results of Examination

The following statement shall be included in all STR reports to indicate which loci were utilized:

> *DNA recovered from the above submitted samples was processed using the polymerase chain reaction (PCR) and the PowerPlex® Fusion system.*
>
> If a sample was previously analyzed with a kit other than PowerPlex Fusion the following statement should be added to the report:
>
> *DNA recovered from ITEM(s) 1 was/were previously analyzed using using the polymerase chain reaction (PCR) and the PowerPlex® lex 16 HS (or other STR kit) system.*

2.10.10.2 No Results Samples

Criteria	Statement
No profile obtained	A DNA profile was not obtained from ITEM 1

2.10.10.3 Male DNA Screening Conclusions:

For sex assault samples that are screened for the presence of male DNA using the Plexor HY quantitation chemistry, the following statement shall be used:

Criteria	Statement
For samples that are positive for male DNA and sent forward for STR testing	DNA recovered from the following indicated the possible presence of male DNA and was processed further. Item Number in list format.
For samples that are positive for male DNA and not sent forward for STR testing	DNA recovered from the following indicated the possible presence of male DNA and was not processed further. Item Number in list format.
For samples that are negative for male DNA and not sent forward for STR testing.	DNA recovered from the following did not indicate the possible presence of male DNA and was not processed further. Item Number in list format.
For samples that are inconclusive for male DNA and sent forward for STR testing.	DNA recovered from the following was inconclusive for the possible presence of male DNA and was processed further. Item Number in list format.
For samples that are inconclusive for male DNA and not sent forward for STR testing.	DNA recovered from the following was inconclusive for the possible presence of male DNA and was not processed further. Item Number in list format.

2.10.10.4 Statistical Reporting:

Criteria	Statement
RMP	Population statistics are offered to estimate the frequency of occurrence of the reported DNA profiles in the general population. The method used applies recommended procedures endorsed by the National Research Council. (ref. The Evaluation of Forensic DNA Evidence 1996). Using available population frequency data, the probability of selecting an unrelated individual at random from the population having a DNA profile matching the profile from ITEM 1 (description) is: Population Database Random Match Caucasian # African American # Hispanic #
CPI	Calculation based on the combined probability of exclusion (CPE) assume the random selection of unrelated individuals from the general population. The calculations in use have been endorsed by the DNA Advisory Board (2/23/00). The CPE is an estimate of the percent of individuals from the general population that would be excluded as donors to the reported DNA mixture. The combined probability of inclusion (CPI) is an estimate of the percent of individuals from the general population that would be possible donors to the reported DNA mixture. The reciprocal of the CPI (1/CPI) is an estimate of selecting an individual from the general population that could have contributed to the reported mixture. The reciprocal CPI from the mixture detected from ITEM 1 (description) is: Population Database Reciprocol CPI Caucasian # African American # Hispanic #

2.10.10.5 Remarks Statements:

Criteria	Statement
CODIS entry, single source sample	The DNA profile from ITEM 1 was entered into the COmbined DNA Index System (CODIS).

CODIS entry, major donor	The DNA profile from the major donor to ITEM 1 was entered into the COmbined DNA Index System (CODIS).
CODIS entry, minor donor	The DNA profile from the minor donor to ITEM 1 was entered into the COmbined DNA Index System (CODIS).
CODIS entry no, sample	The DNA profile from ITEM 1 was not entered into the COmbined DNA Index System (CODIS).
CODIS entry no, case	DNA profiles were not entered into the COmbined DNA Index System (CODIS).
Reference sample request	Please submit a known buccal swab collected from potential donors to this case to be used for further analysis and comparisons.
Reagent blank types present	A negative control associated with this case did not perform as expected. It did not affect the interpretation of the results.
Positive control performance failure	A positive control associated with this case did not perform as expected. It did not affect the interpretation of the results.
Paternity reference samples	The DNA profiles obtained will be evaluated further for potential paternity and/or relationship testing. The results of this evaluation will be the subject of a separate report.
Y-STR, submitted	ITEM(S) 1 has/have been submitted for Y-STR testing. The result of that examination will be the subject of a separate report.
Y-STR, eligible	ITEM(S) 1 may be eligible for Y-STR testing if a suspect reference sample is submitted for comparison purposes. Please contact the analyst listed below for additional information, if needed.
Prior mixtures used for exclusion only	Updated laboratory policy does not allow for comparison of minor DNA types of complex mixtures for exclusionary purposes.
Additional staff involved in FA Case Record	Other staff members involved in FA Case RecordOther members of the Forensic Science Division may have processed evidence associated with this report, in addition to the reporting analyst.

2.10.11 Interpretation of profiles associated with criminal paternity

Trio Parentage-Cases requesting comparison between a known parent, known child/product of conception and an alleged parent may be termed a trio parentage case. Conclusions on these types of cases shall only be developed if all three samples are available for testing. The DNA profile developed from a product of conception/child should first be compared to the known parent's DNA profile to determine obligate alleles for the alleged parent. Obligate alleles are those that must have been donated by the alleged parent. The obligate alleles may then be compared to the alleged parent. If the alleged parent's DNA profile contains the obligate alleles, the alleged parent cannot be excluded as a parent. If the alleged parent's DNA profile does not contain the obligate alleles, the alleged parent is excluded as a possible parent. It is not uncommon for a product of conception/child and biological parent to have one or more loci with an allele other than the obligate allele due to mutation. In general, exclusions should only be declared when three or more loci do not have concordant obligate alleles with the alleged parent. These instances must be evaluated with caution and the mutation frequency for the particular locus in question considered.

Single parent cases involve a single alleged parent and a known child, without the aid of a second known parent. These types of cases are commonly referred to as a single parent reverse paternity scenario. If the alleged parent's DNA profile contains alleles in common at each genetic marker when compared to the child's DNA profile, an inclusion is reported. If the alleged parent's DNA profile does not contain alleles in common at each genetic marker when compared to the child's DNA profile, an exclusion is reported. It is not uncommon for a child and biological parent to have one or more loci with an allele not in common with a biological parent due to mutation. In general, exclusions should only be declared when three or more loci do not have concordant obligate alleles with the alleged parent. These instances must be evaluated with caution and the mutation frequency for the particular locus in question considered.

Standard reverse paternity cases typically involve two parents and an unknown child. If both parents' DNA profiles contain alleles in common at each genetic marker when compared to the child's DNA profile, an inclusion is reported. If both parents' DNA profiles do not contain alleles in common at each genetic marker when compared to the child's DNA profile, an exclusion is reported. It is not uncommon for a child and biological parent to have one or more loci with an allele not in common with a biological parent due to mutation. In general, exclusions should only be declared when three or more loci do not have concordant obligate alleles with the alleged parent. These instances must be evaluated with caution and the mutation frequency for the particular locus in question considered.

Cases submitted for testing that are not analytically processed by MSP shall either be outsourced to a private vendor laboratory through backlog reduction programs or returned to the submitting agency for submission to a private vendor laboratory.

Paternity Scenarios

Criteria	Statement

1 known parent and 1 known child/POC, 1 alleged parent included (Standard Trio)	Based on the DNA profiles obtained for ITEM 1 (known parent) and ITEM 2 (alleged parent), the alleged mother/father cannot be excluded as the possible biological mother/father of ITEM 3 (child/POC). It is approximately ### times more likely that ITEM 2 (alleged parent) is the biological parent of ITEM 3 (child/POC) than an untested, randomly selected individual in the (population) that is unrelated to ITEM 3 (child/POC). *Repeat for each population.*
1 known parent and 1 known child/POC, 1 alleged parent excluded (Standard Trio)	Based on the DNA profiles obtained for ITEM 1 (known parent) and ITEM 2 (alleged parent), the alleged mother/father is excluded as the possible biological mother/father of ITEM 3 (child/POC).
1 alleged parent and 1 known child/POC, inclusion (Single Parent)	Based on the DNA profile obtained for ITEM 1 (child/POC), ITEM 2 (alleged parent) cannot be excluded as the possible biological parent of ITEM 1 (child/POC). It is approximately ### times more likely that ITEM 2 (alleged parent) is the biological parent of ITEM 1 (child/POC) than an untested, randomly selected individual in the (population) that is unrelated to ITEM 1 (child/POC). *Repeat for each population.*
1 alleged parent and 1 known child/POC, exclusion (Single Parent)	Based on the DNA profile obtained for ITEM 1 (child/POC), ItEM 2 (alleged parent) is excluded as the possible biological parent of ITEM 1 (child/POC).
1 alleged child/POC, 2 parents, inclusion (Standard Reverse)	Based on the DNA profile obtained for ITEM 1 (alleged child/POC), the alleged child cannot be excluded as the possible biological child of ITEM 2 (parent 1) and ITEM 3 (parent 2). It is approximately ### times more likely that ITEM 1 (alleged child/POC) is the biological child ot ITEM 2 (parent 1) and ITEM 3 (parent 2) than an untested, randomly selected individual in the (population) that is unrelated to ITEM 1 (alleged child/POC). *Repeat for each population.*
1 alleged child/POC, 2 parents, exclusion (Standard Reverse)	Based on the DNA profile obtained for ITEM 1 (alleged child/POC), the alleged child is excluded as the possible biological child of ITEM 2 (parent 1) and ITEM 3 (parent 2).

2.10.12 Statistics

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Budowle B et.al. Core STR Loci Data from 41 Sample Populations. J For Sci 2001. 46(3), pp. 453-489.

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Federal Bureau of Investigation. CODIS User's Guide. Chapter 8, PopStats. 1997a.

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Kristen Lewis O'Connor, Carolyn R. Hill, Peter M. Vallone, John M. Butler (2011) Linkage disequilibrium analysis of D12S391 and vWA in US population and paternity samples. *Forensic Sci. Int. Genet*. 5: 538-540.

2.10.12.1

The minimum allele frequencies are calculated using 5/2N where N is the number of individuals in the population database. This follows the NRC II recommendation for STR analysis.

2.10.12.2

Off-ladder alleles may be used to determine a match and estimate the significance of a match. The allele frequency will be the calculated minimum allele frequency for the locus/population group.

2.10.12.3

Tri-allelic loci that have been confirmed by re-amplification will be used to determine a match, but will not be used for the statistical estimation.

Due to the potential for linkage disequilibrium for genetic markers D12S391 and vWA, the least significant marker should be dropped from calculations when close relatives are included within a mixture.

2.10.12.4

The FBI's PopStats software will be configured to use the formula listed below to calculate significance estimates.

Single source/deduced profile calculations will use NRC II Recommendation 4.1 at a given locus and may be performed using PopStats.

For heterozygotes: f=2pq

For homozygotes: f=p2+p(1-p) where equals 0.01

Overall profile frequency F=(f1xf2xf3xf4x...fk) where k is the number of loci.

Mixture profile calculations will include all loci where a reference sample was included and all alleles have been detected (exceeded the Stochastic Threshold). Loci that were inconclusive, not detected or contained the potential for allelic dropout (DNA alleles below the Stochastic Threshold) shall not be used in calculations. PopStats may be used to perform the calculations.

For each locus, the frequency of alleles detected at a locus P=(p1+p2+p3+p4+pk)2 where p is the estimated frequency of each individual allele detected for allele 1 through k where k is the number of alleles at the locus.

For each locus, the frequency of alleles not detected Q=1-P

The probability of exclusion at each locus PE=Q2+Q(1-Q)+2Q(1-Q)(1-) where equals 0.01

The combined probability of exclusion CPE=1-[(1-PE1)(1-PE2...(1-PEk)] where k is the number of genetic loci.

The combined probability of inclusion CPI=1-CPE

The inverse of the CPI, or 1/CPI shall be reported.

A Parentage Index may be calculated and reported in standard triad paternity cases using PopStats. A genetic locus that includes a potential mutation will require input of the mean Power of Exclusion (Budowle et.al. 1999) and paternal mutation rate (http://www.cstl.nist.gov/biotech/strbase/mutation.htm). Genetic locus D12S391 shall not be utilized in Parentage Index calculations due to potential linkage with vWA.

2.10.12.5

PopStats-Click the desired case type button on the PopStats toolbar. The window will display the selected case type. Type a Specimen ID in the Reference field. Verify that all of the included loci have an "x" to the left of the locus name in the Locus column. Deselect the box for loci not utilized. Type valid alleles in the target profile grid(s). Click Calculate Statistics.

2.10.12.6

PopStats will be configured to generate statistical estimates using the Caucasian, African American, Hispanic populations.

For RMP calculations, the Caucasian, African American and Hispanic populations will be included within the laboratory report. All requests for case-specific population databases shall be forwarded through the Technical Leader.

2.10.12.7

A Random Match Probability shall be generated quarterly for the Internal Laboratory Control, maintained and reviewed by a supervisor to ensure settings within PopStats have not been altered. The schedule of the quarterly check will appear on two staff members calendars to ensure the check is completed during that quarter.

2.10.13

Per FBI Quality Assurance Standards, deviations from the Interpretational Guidelines may only be approved by the DNA Technical Leader.

2.11 Interpretational Guidelines for AB 3500/3500xL STR Profiles- PowerPlex® Fusion and STRMix

2.11 Interpretation Guidelines for AB 3500/3500xL STR Profiles-PowerPlex® Fusion and STRMix™

These procedures describe the method by which nuclear DNA typing results are verified and interpreted for forensic comparison purposes. Nuclear DNA typing results are obtained from the PowerPlex® Fusion amplification kit using the GeneMapper™ IDx DNA typing software. Interpretation and statistical analysis is performed using STRMix™.

Verification and evaluation of ladders, controls and reagent blanks do not need to be repeated when they are the subject of a separate Forensic Advantage record number that has successfully passed a DNA technical review.

2.11.1 Step 1: INTRODUCTION

Short Tandem Repeats, or STRs, are genetic markers that contain short repeated sequences of DNA base pairs. These repeated sequences are typically in the 3-7 base pair size range. They are distributed throughout the human genome and represent a good source to differentiate individuals. For this reason, STRs are said to be highly polymorphic. STRs may be detected through the use of a combination of techniques including the Polymerase Chain Reaction (PCR) and capillary electrophoresis. The PCR process amplifies a specific location (locus) on the chromosome utilizing fluorescently labeled primers and the enzyme *Taq* Polymerase. Capillary electrophoresis separates the DNA fragments by size, allowing for allele designations. The allele designation is based upon the number of repeat sequences in the DNA fragment. Autosomal STR genotypes represent DNA types from both maternal and paternal origins.

The PowerPlex® Fusion chemistry kit allows for the amplification of 22 autosomal STR genetic markers plus the Amelogenin (gender) locus and a Y-chromosome STR. The autosomal STR genetic markers include D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA and D22S1045. The Y-chromosome STR is DYS391. The PowerPlex® Fusion kit utilizes a five color detection system. Fluorescein (FL) is utilized for Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317 and Penta E. The dye set JOE is utilized for D16S539, D18S51, D2S1338, CSF1PO and Penta D. TMR-ET is utilized for TH01, vWA, D21S11, D7S820, D5S818, TPOX and DYS391. The dye set CXR-ET is utilized for D8S1179, D12S391, D19S433, FGA and D22S1045. All of the loci are co-amplified in a single reaction, or tube, and separated by a single electrokinetic injection on an ABI 3500/3500xL electrophoresis genetic analyzer utilizing Promega's CC5 Internal Lane Standard 500 sizing standard. Data analysis can be accomplished using ABI's GeneMapper® ID-X v. 1.4 software in combination with Promega's PowerPlex® Fusion bins and panels set.

A summary of each locus within the PowerPlex® Fusion amplification kit and the designation are displayed below:

Genetic Marker	Min. Size (bp)	Max. Size (bp)	Repeat Structure	Ladder Alleles
Amelogenin	89	95	N/A	X,Y
D3S1358	103	147	4	9-20
D1S1656	161	208	4	9-14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D2S441	214	250	4	8-11, 11.3, 12-17
D10S1248	256	280	4	8-19
D13S317	302	350	4	5-17
Penta E	371	466	5	5-24
D16S539	84	132	4	4-16
D18S51	134	214	4	7-10, 10.2, 11-13, 13.2, 14-27
D2S1338	224	296	4	10, 12, 14-28
CSF1PO	318	362	4	5-16
Penta D	377	450	5	2.2, 3.2, 5-17
TH01	72	115	4	3-9, 9.3, 10-11, 13.3
vWA	127	183	4	10-24
D21S11	203	259	4	24, 24.2, 25, 25.2, 26-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2 36-38
D7S820	269	313	4	5-16
D5S818	321	369	4	6-18
TPOX	393	441	4	4-16
DYS391	442	486	4	5-16
D8S1179	76	124	4	7-19
D12S391	133	185	4	14-17, 17.3, 18, 18.3, 19-27
D19S433	193	245	4	5.2, 6.2, 8-12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2

FGA	265	411	4	14-18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26-30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
D22S1045	425	464	3	7-20

2.11.2 Step 2: GeneMapper® ID-X v. 1.4 of PowerPlex® Fusi on 3500/3500xL Genetic Analyzer Data

2.11.2.1

Open the GeneMapper® ID-X v1.4 software by logging into the software as DNA User. Import the sample files from the 3500/3500xL Genetic Analyzer project file into a GeneMapper® ID-X v1.4 project by selecting Edit>Add Samples to File from the toolbar.

2.11.2.2

Select the appropriate sample type, analysis method (i.e. Fusion_3500), panel and size standard in the corresponding column and drop-down box. The analysis method incorporates a 250 RFU threshold for peak detection and a 75 RFU threshold for ILS peak detection.

2.11.2.3 Allele Declaration

Alleles are declared when they meet the following criteria

The allele is within a ± 0.5-bp "window" around the size obtained for the corresponding allele in the Allelic Ladder.

The peak height is 250 RFUs (Relative Fluorescent Units). The 3500/3500xL Genetic Analyzer Analytical Threshold (AT) is 250 RFUs. The analytical threshold is defined as the minimum peak height in RFUs for assigning an STR type to an observed PCR peak. It is set based upon instrument sensitivity, baseline noise and amplification chemistry . Peaks observed within electropherograms that do not meet or exceed the analytical threshold of 250 RFUs are not labeled by the 3500/3500xL Genetic Analyzer using GeneMapper® ID-X v. 1.4 software and are not interpreted.

2.11.2.4

Select Analyze, or the green arrow. Save the project by batch starting with analyst initials_date of run at a minimum. Move the project to the designated location for quarterly backup.

2.11.2.5

Initially, labels from peaks shall not be removed manually during review of electropherograms within GeneMapper® ID-X v1.4, but may be annotated with additional relevant information. For instance, labels associated with peaks clearly resulting from a fluorescent spike cannot be removed but an annotation should be added either within GeneMapper® ID-X v1.4 or by another means. If adding labels to peaks within GeneMapper® ID-X v1.4, you may click on the label box/peak to highlight and then right click on peak. Select Rename Allele and then select Custom. Type in any additional information and click OK. Upon review, print electropherograms or utilize electronic capture methods.

2.11.2.6

Export the project into the designated location for backup by clicking Tools and then GeneMapper® Manager. Alternatively, depress the Control button and M at the same time (Ctrl+M). Select project from list and click Export. Select run folder, enter in project's name, and click Save. Click Done to exit out of GeneMapper® Manager. Electronic data shall be stored on an external electronic medium (i.e. CDs) at least quarterly and maintained.

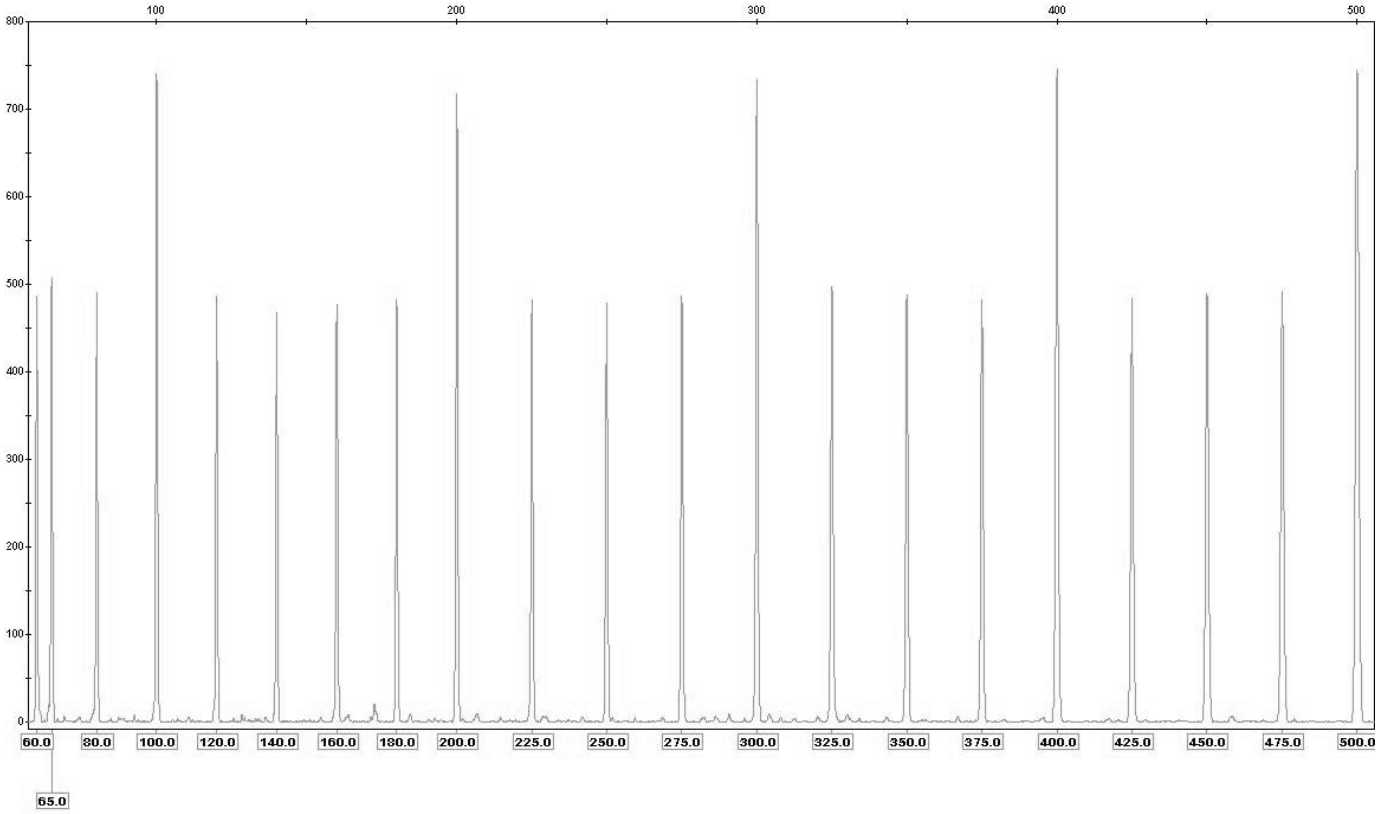
2.11.2.6

At any time, raw data may be viewed in GeneMapper® ID-X v1.4 by clicking View then Raw Data or by depressing the Control button and F2 at the same time (Ctrol+F2).

2.11.3 Step 3: EVALUATION OF ILS AND ALLELIC LADDERS

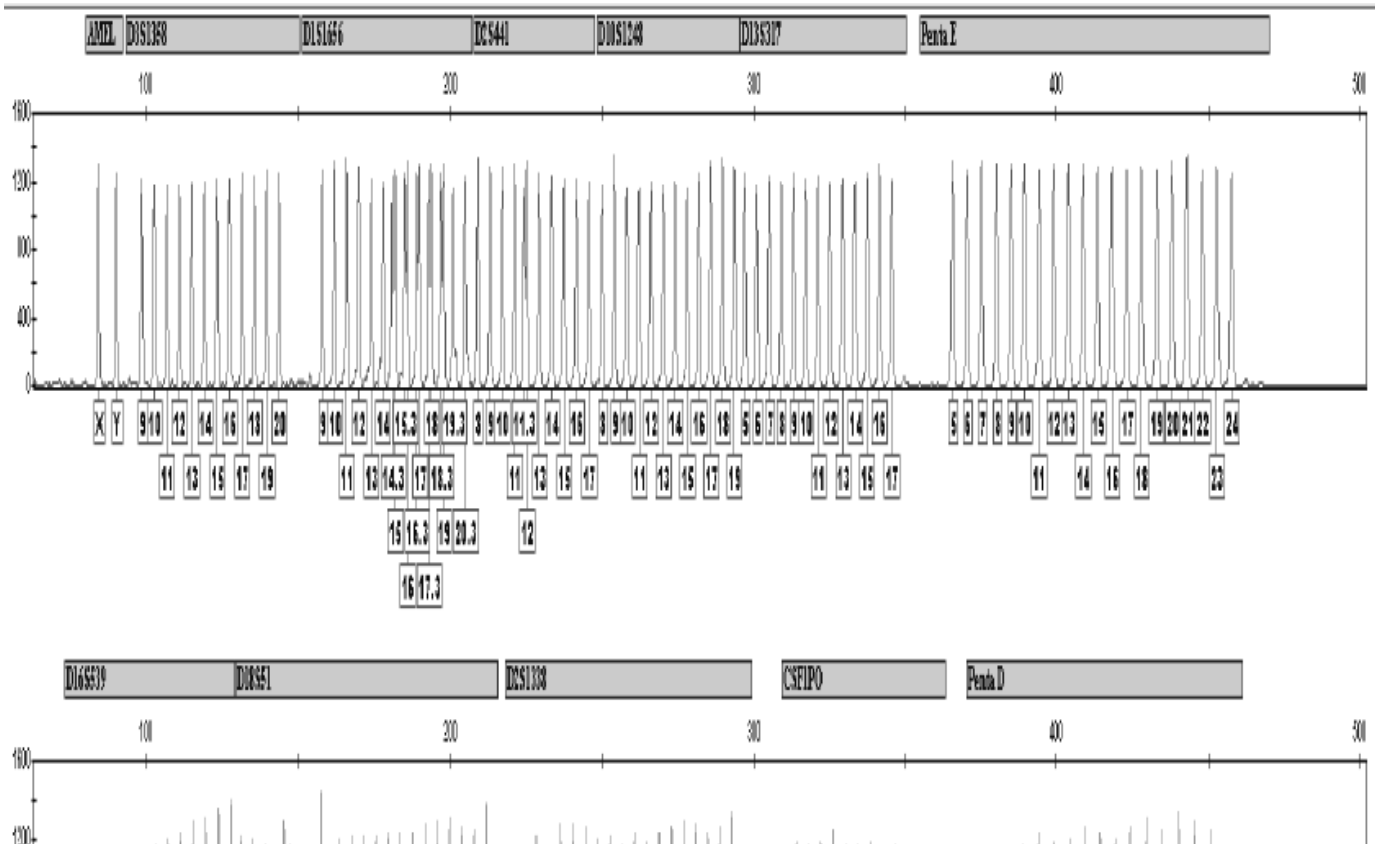
2.11.3.1 Internal Size Standard

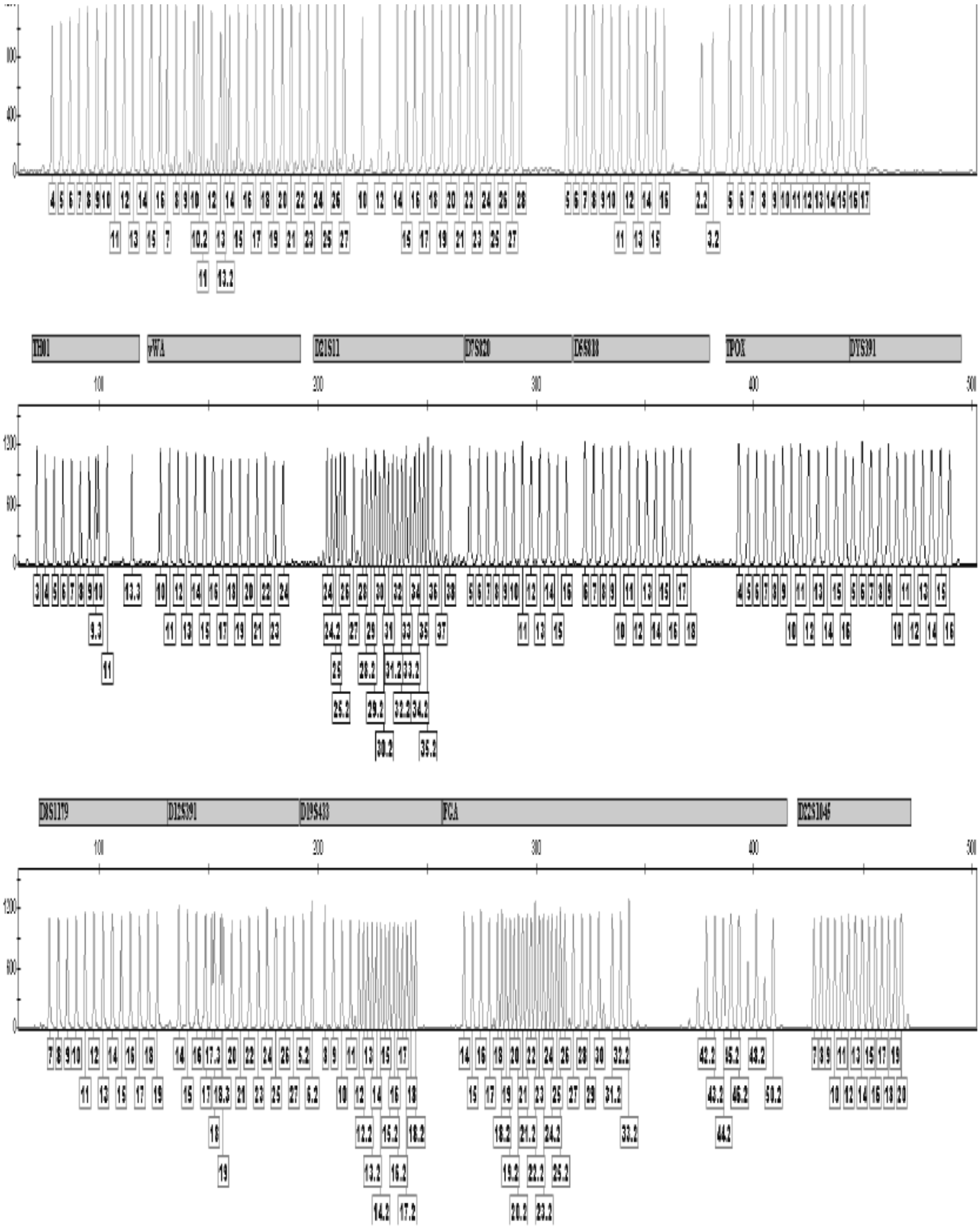
The Internal Lane Standard (ILS 500) shall be examined to verify that the fragments have been properly labeled as depicted below. All size standard peaks should be greater than or equal to the analytical threshold of 75 RFUs. ILS 500 peaks that do not exceed the 75 RFUs analytical threshold should be evaluated with caution, ensuring that the low RFUs are not indicative of the overall performance of the detection and amplification.



2.11.3.2 Allelic Ladder

The Allelic Ladder shall be examined to verify that the types have been properly assigned as depicted in the allelic ladder below and that all alleles are greater than or equal to 250 RFUs.





2.11.4 Step 4: Artifact Declaration

Review electropherograms for each sample amplified to identify artifacts and overall run quality. This may be accomplished by highlighting

samples and clicking the Display Plots button under the Analysis menu. Alternatively, depress the Control button and L at the same time (Ctrl+L).

The position of the artifact or anomaly in relation to the allelic ladder should be considered in each instance. Both the Raw Data and GeneMapper ® ID-X v. 1.4 data should be evaluated to make the interpretation of the artifact or anomaly. The characteristics of the artifact or anomaly should be carefully evaluated before changes in injection time, dilution adjustment or re-amplification is initiated.

STUTTER

Stutter peaks are a common artifact associated with the amplification of DNA using the Polymerase Chain Reaction and are due to strand slippage of the enzyme Taq Polymerase. Stutter peaks are often observed one repeat unit less than the actual allelic peak (N-3, N-4, N-5), and to a lesser extent one repeat larger than the actual allelic peak (N+3, N+4, N+5). Within a genetic locus, larger alleles may give stutter peaks that are larger than smaller alleles. Note: The major peak must not have indications of over-saturation of the genetic analyzer detector. If the main peak is over-saturated in a reference sample and an allele is declared in a stutter position, the sample must be re-run or re-amplified to obtain a main peak that does not show indications of over-saturation.

If the peak height ratio is greater than or equal to the stutter threshold the allele is declared. It should be noted that a perceived N-3, N-4 or N-5 stutter peak may slightly exceed the established stutter threshold dependent upon a number of factors such as stochasm. One of the instances whereby the stutter percentage may exceed the established threshold occurs when a heterozygous pair of alleles are separated by 2 repeat units. The presence of a negative stutter peak for the larger allele, combined with a positive stutter peak from the smaller allele, may cause the artifact to exceed the established threshold. This observation should be evaluated cautiously, taking the full profile and the presence of additional minor contributors into consideration.

If the peak height ratio is less than the stutter threshold, the allele is not declared.

Genetic Locus	Negative Stutter (one repeat)
D3S1358	≤ 11.9%
D1S1656	≤ 14.2%
D2S441	≤ 9.2%
D10S1248	≤ 12.4%
D13S317	≤ 9.8%
Penta E	≤ 7.6%
D16S539	≤ 10.2%
D18S51	≤ 14.6%
D2S1338	≤ 13.9%
CSF1PO	≤ 9.5%
Penta D	≤ 6.8%
TH01	≤ 4.6%
vWA	≤ 11.2%
D21S11	≤ 11.6%
D7S820	≤ 11%
D5S818	≤ 9.5%
TPOX	≤ 5.5%
DYS391	≤ 8.7%
D8S1179	≤ 10.9%
D12S391	≤ 15.8%
D19S433	≤ 11%
FGA	≤ 12.1%
D22S1045	≤ 16.4%

Amelogenin is not an STR genetic marker, therefore, it does not have the same tendency to produce a stutter peak. Genetic locus D22S1045 tends to produce positive stutter at a higher level than other markers within the Fusion kit and has a positive stutter threshold of 8.6%. Additionally, genetic locus D1S1656 has an N-2 stutter threshold set at 3.6%.

Variant - (Off-ladder Alleles)

A tetra-nucleotide repeat STR will have allele names that differ by four base pairs and penta-nucleotide repeats will vary by five base pairs, and so on. Microvariants differ from common alleles by one or more base pairs (less than the consensus repeat unit). Because microvariants and

some rare alleles often do not label the same as consensus alleles present in the reference allelic ladder, they are often referred to as "off-ladder" alleles. GeneMapper® ID-X v. 1.4 incorporates into the bin set many of the common microvariants observed. If an off-ladder allele is not recognized by GeneMapper® ID-X v. 1.4, it can be assigned an allele name by calculating the number of base pairs separating it from the nearest lower allele in the reference allelic ladder. The number of base pairs is added to the allele call of the nearest lower allele separated by a decimal. For instance, an allele at 257.51 base pairs is labeled in GeneMapper® ID-X v. 1.4 as an off-ladder allele. The next nearest lower allele is a 28 at 256.64 base pairs in size. The difference is near 1 base pair. The allele is designated 28.1. The migration differences in the sample as compared to the reference allelic ladder may be considered in determining the base pair size difference. Reference the appropriate section of this document regarding any requirements for re-amplification of "off-ladder" alleles.

Virtual alleles falling above the largest or below the smallest "real" peak of the allelic ladder may not be recognized for CODIS entries. See Biology Procedure 2.17 for a listing of alleles eligible to be entered into CODIS.

Alleles designated "OL" should be evaluated based on the base pair size and recorded as previously noted. Any subsequent comparison of these "OL" alleles will also be based on the base pair size.

The minimum allele frequency for the locus will be used in the statistical estimation calculation.

Confirmation of off-ladder alleles and tri-allelic patterns

Off-ladder alleles and tri-allelic patterns observed in samples used for current or future inclusionary purposes or CODIS entry (off-ladder alleles only) may be confirmed by re-amplification when sample is available. In the case of multiple samples exhibiting the off-ladder allele or tri-allelic pattern amplified on the same thermal cycler and at the same time, only one sample of the amplification set may be re-amplified. In the case of multiple samples exhibiting the off-ladder allele or tri-allelic pattern amplified on separate occasions, re-amplification is not required.

Off-ladder alleles observed in reference samples, where the reference sample is excluded as a potential donor based on multiple loci or evidentiary profiles were not observed on the case, may not require confirmation.

Incomplete "A" nucleotide addition

An allele may display a leading peak shoulder approximately one base pair shorter than the allele with which it is associated. This is a result of incomplete adenine addition during the final extension cycle. This phenomenon can be associated with increased target DNA or the presence of *Taq* Polymerase inhibitors.

Alleles exhibiting minor shoulders in which the peak base is narrow and the major peak is sharp and well defined may be called without further processing.

If the shoulder peak interferes with the interpretation and/or exhibits major shoulders and broad peak bases, the sample should be re-amplified at a lower concentration or further manipulated to reduce/eliminate the effects of inhibition.

Notation

The incomplete "A" nucleotide addition should be documented with the base pair sizes and noted on the electropherogram as "-A".

Amplification inhibition

Amplification of genetic markers utilizing the Polymerase Chain Reaction (PCR) is an enzymatic reaction. Certain conditions and/or substances may inhibit the reaction. The results of inhibition may generally be noted at two steps during the analytical process, qPCR DNA quantitation and STR detection.

DNA samples exhibiting inhibition at the quantitation stage may show an increase in Ct values and/or lack of IPC amplification. Samples exhibiting inhibition should be evaluated cautiously. Dependent upon sample type, quantity/volume and qPCR data, the sample may be re-extracted, diluted or re-phenol-ed to remove the inhibitor prior to re-quantification and/or amplification for STRs.

DNA samples exhibiting inhibition at the STR detection stage typically show an inhibition "pattern" that may include an overall reduction in allelic RFUs and/or a lack of sensitivity at genetic loci with larger base pair sizes. Samples exhibiting inhibition effects for STR amplification with PowerPlex® Fusion typically show a reduced RFU level for the Amelogenin marker. DNA samples exhibiting inhibition at the STR detection stage may be re-extracted, diluted or re-phenol-ed to remove the inhibitor prior to re-amplification to remove the effects of the inhibitor. Dependent upon the option selected, the sample(s) may need to be re-quantified.

Elevated Stutter Peaks

Single Source Samples (Known)-

Stutter outside the defined range of stutter for a single source sample should be evaluated with respect to the entire profile. If it is judged that the sample profile is characterized by good balance at all of the loci the sample does not need to be rerun.

If the sample does not show good balance then the sample should be re-run or re-amplified.

Notation: Note the stutter percentage on the electropherogram.

Forensic Unknowns

Samples in which the overall profile is consistent with a single source origin, but a peak(s) positioned at the stutter position (n+4, n-4 or n-5 dependent upon the locus repeat motif) exceeds the stutter criteria and reporting threshold, the sample may be re-amplified. If the peak(s) is reproducible upon re-amplification, or if the sample is not re-amplified, then the reported conclusion should indicate that the n+4, n-4 or n-5 peak could be attributed to either a triallelic pattern, possible additional donor(s) or an artifact (elevated stutter).

Samples in which the overall profile is consistent with a mixed sample, elevated stutter should not be considered.

Notation

Note the stutter percentage on the electropherogram.

Fluorescent Pull-Up

Fluorescent pull-up occurs when a peak on an electropherogram, represented by a DNA type, has a sufficiently high RFU value that the matrix/spectral is unable to compensate for the overlapping color fluorescence. This produces a peak in a subsequent color(s) with the same, or nearly the same, base pair size as the peak represented by a true DNA type.

Fluorescent pull-up must be documented on the electropherogram with the base pair sizes of the peaks involved and a notation indicating pull-up.

Electropherograms that contain a possible pull-up peak labeled by GMID-X v. 1.4 as an OL allele may be re-injected or re-amplified to remove the artifact or labeled as a "pull-up" peak. Electropherograms that are indicative of a single source profile that contain a peak deemed as a possible pull-up peak but is labeled by GMID-X v. 1.4 as an allele may be re-injected or re-amplified to remove the artifact or labeled as a "pull-up" peak. Electropherograms that are indicative of a mixed donor profile that contain a peak deemed as a possible pull-up but is labeled by GMID-X v. 1.4 as an allele shall be re-injected or re-amplified to remove the artifact.

Note "pull-up" on the electropherogram.

Fluorescent Spiking

A fluorescent spike occurs due to the presence of a particle and/or bubble within the capillary. A peak(s) is generated on the electropherogram due to the generation of fluorescence from the laser as it interacts with capillary bubbles and/or particles.

If the spike is observed in the same position in two or more dyes in the electropherogram, the sample does not have to be rerun. For instances whereby the fluorescent spike falls between bins and cannot be added to the peak label within GeneMapper ID-X v. 1.4, the base pair sizes can be determined by placing the cursor over the peak. The base pair size shall be noted in the case file as supporting documentation.

A fluorescent spike may be observed in a single color in very rare instances. Typically the peak morphology is not consistent with peaks represented by DNA alleles. A single color fluorescent spike that is >275 RFUs shall be re-injected to distinguish between low intensity alleles and fluorescent artifacts. If the artifact is removed, the interpretation may proceed. If the artifact is reproduced the peak should be re-evaluated as a potential DNA allele.

Notation

Note on the electropherogram or within the case file "fluorescent spike" or "f-spike", with base pair size noted.

Pull-up

Single source samples (Known)

If the sample exhibits good balance and well-formed peaks the sample does not need to be rerun. Samples with detector oversaturation shall be re-injected or re-amplified to achieve lower RFUs.

If the sample exhibits exaggerated stutter peaks, exaggerated minus "A" and flat-lined or inverted peaks the sample must be re-injected or re-amplified.

Notation

Note on the electropherogram "possible over-saturation".

Forensic Unknown

The sample should be rerun to bring the peak height down to an RFU level that does not exhibit oversaturation. If the sample rerun is accompanied with unbalanced conditions, the sample should be re-amplified. If an allele is unable to be reduced below saturation, the locus should be reported as inconclusive.

Loci reported as inconclusive shall include a notation of "inc" on the electropherogram.

Amelogenin peaks exceeding saturation in known reference and evidentiary samples don't need to be re-injected at lower injection times and no notation is necessary.

Raised Sample Baseline

A sample that displays excessive raised baseline should be re-injected using the standard injection time or for a reduced time. If such subsequent injections do not result in on-scale data, the sample may be re-amplified with less DNA template.

Excessive DNA Template and Off-Scale Samples

An excessive amount of template DNA may result in the appearance of off-scale peaks, which exceed the linear dynamic range of the genetic analyzer detector. These samples may also exhibit raised baseline, pull-up, -A, atypical stutter and/or non-specific peaks. These samples may

be re-injected for reduced time, re-amplified with less input DNA template and/or re-extracted. If the sample profile is to be used in a STRMix interpretation, it is important that the data be free from excessive artifacts. Re-extraction, re-amplification or re-injections may be conducted to remove excessive artifacts over removing them from the data prior to STRMix evaluation.

Additional Artifacts

Additional artifacts may be recognized during interpretation such as alleles associated with non-human sources, raised baseline or non-allelic peaks associated with fluorescent dyes. These artifacts should be interpreted with caution and supporting documentation (manufacturer's communications, literature etc...) included within the case file.

2.11.5 Step 5: EVALUATION OF POSITIVE AND NEGATIVE CONTROLS

Positive Amplification Control (2800M)

Purpose: To provide a documented positive human DNA control to monitor the amplification process. The Positive Amplification Control is run with each amplification set and kit.

The Positive Amplification Control profile is:

Genetic Locus	2800M Genotype
Amelogenin	X,Y
D3S1358	17,18
D1S1656	12,13
D2S441	10,14
D10S1248	13,15
D13S317	9,11
Penta E	7,14
D16S539	9,13
D18S51	16,18
D2S1338	22,25
CSF1PO	12,12
Penta D	12,13
TH01	6,9.3
vWA	16,19
D21S11	29,31.2
D7S820	8,11
D5S818	12,12
TPOX	11,11
DYS391	10
D8S1179	14,15
D12S391	18,23
D19S433	13,14
FGA	20,23
D22S1045	16,16

Internal Laboratory Control (ILC): NIST-Traceable Standard

Purpose: The Internal Laboratory Control originated as an unknown male whole blood sample preserved in K_3-EDTA. Bloodstains were prepared from the whole blood on filter paper. The Internal Laboratory Control was analyzed against the NIST SRM 2391C, making it a NIST-traceable standard.

The Internal Laboratory Control is intended to provide a documented positive human DNA control to monitor the complete DNA analytical process. The Internal Laboratory Control is run with each case, or batch of cases, and is processed with the known samples (reference DNA samples). An Internal Laboratory Control sample shall be included for each stain extraction method utilized (Tissue/Hair or Stain Extraction Buffer) for a case or batch of cases. The Internal Laboratory Control is amplified with each amplification kit and the same thermal cycler model as

the samples it is controlling. Additionally, the Internal Laboratory Control is run on each Genetic Analyzer model utilized in each case, or batch of cases it controls. It is acceptable to extract the Internal Laboratory Control with the evidentiary samples only for instances that a batch and/or case do not contain known samples or the known samples are extracted with a different extraction procedure with their own Internal Laboratory Control sample. An Internal Laboratory Control does not need to be included in batches that only contain differential extraction types.

The Internal Laboratory Control (ILC) profile is:

Genetic Locus	ILC Genotype
Amelogenin	X,Y
D3S1358	14,16
D1S1656	14,16
D2S441	13,15
D10S1248	15,17
D13S317	11,12
Penta E	8,16
D16S539	11,11
D18S51	12,21
D2S1338	19,20
CSF1PO	8,10
Penta D	2.2, 2.2
TH01	7,7
vWA	13,16
D21S11	30,33.2
D7S820	10,12
D5S818	11,11
TPOX	11,11
DYS391	11
D8S1179	13,14
D12S391	18,23
D19S433	14.2,15
FGA	24,24
D22S1045	14,15

Positive Control Performance Criteria - (2800M and Internal Laboratory Control)
Genotypes must match the expected genotype at each locus. The relative fluorescent intensity must meet the minimum analytical threshold of 250 RFUs. Exceptions to meeting the minimum analytical threshold of 250 RFUs are outlined below. Because the 2800M and Internal Laboratory Control samples are single source in nature, it is acceptable to have homozygous alleles below the 900 RFUs Stochastic Threshold. It is acceptable for the 2800M and Internal Laboratory Control samples to have Peak Height Ratios (PHRs) less than the expected 50% for heterozygous markers. The 2800M and Internal Laboratory Control controls are single source samples. Alleles with very high RFUs that exhibit potential oversaturation of the genetic analyzer detector as represented by flat peak tops and/or associated elevated stutter peaks may be interpreted if the peaks are symmetrical, sharp and potential artifacts do not interfere with the interpretation of the electropherogram. Alleles that do exhibit oversaturation as described previously must be reanalyzed by reamplification and/or reinjection at reduced injection conditions.

Performance failures of Positive Controls

Incorrect genotypes - If any positive control (2800M or Internal Laboratory Control) has genotypes that are not the expected genotypes at all genetic markers, all samples within the batch must be re-amplified with the same kit initially used. A different lot of the amplification kit may be used if the initial lot is no longer available. Results from samples that do not have sufficient material to re-amplify are invalid and shall not be interpreted or reported.

If any positive control (2800M or Internal Laboratory Control) has alleles at some or all loci that do not meet the 250 RFUs analytical threshold, the entire amplification set shall be evaluated to see if the trend is observed across all samples within the batch. If there is a trend that the entire run is showing characteristics exhibited in the positive control, the amplification is voided and the set shall be re-amplified. If the second positive control associated with the amplification run (Internal Laboratory Control or 2800M) meets expected standards and the over-all characteristics of the run do not mirror the performance of the failed control (RFUs values lower than anticipated), the data can be reported.

Instances where the positive control performance did not meet acceptable standards (greater than or equal to 250 RFUs and/or expected genotype), a statement in the report shall be included indicating the positive control performance failure. Instances where additional action is taken to achieve acceptable positive control performance (increased injection time, re-amplification of the ILC etc...), may be reported without a statement in the report.

Negative Controls

Purpose: To provide a documented control to monitor reagents, test environment and processing procedures associated with the complete PCR analytical process. All negative controls shall correlate with the most restrictive samples in the DNA batch such as extract recovery volume, amplification volume and electrophoresis injection times. All negative controls shall be amplified on the same thermal cycler model and analyzed on the same genetic analyzer model as the samples they are controlling. All extraction sets where potential additional testing using chemistries or testing conditions necessitate additional amplifications shall include two reagent controls for each extraction type. The replicate with the highest DNA concentration for the amplification chemistry being utilized (autosomal-STR or Y-STR) shall by continued through STR typing. The replicate with the lowest DNA concentration shall be archived for possible future additional testing. If both replicates of the reagent control result in an undetected DNA concentration, one reagent control is archived and the other is carried forward to STR amplification.

An EXTRACTION SET is defined as a set of samples and/or controls that are extracted concurrently, using the same extraction procedure and reagent lot numbers. Evidentiary and reference DNA sample sets requiring separation by time and/or space shall have their own extraction reagent blank(s).

STAIN REAGENT CONTROLS - A blank control that is processed with the stain extraction, Chelex extraction or Promega's Tissue and Hair Extraction Kit procedure.

The Stain Reagent Control is set up with each of the stain extraction sets.

DIFFERENTIAL REAGENT CONTROL - A blank control that is processed with the differential extraction procedure.

The Differential Reagent Control is set up with each differential extraction set.

AMPLIFICATION NEGATIVE CONTROL - A blank control that is processed with the amplification procedure.

The Amplification Negative Control is set up with each set of amplifications run concurrently on the same thermal cycler. The Amplification Negative Control should be comprised of the same solution (water or TE^{-4}) that is used in the normalization of DNA samples within the amplification set.

Negative Controls - Performance Criteria

Alleles should not be observed in these samples above the 250 RFUs reporting threshold (analytical/detection).

Peaks greater than 75 RFUs and corresponding to allele positions should be evaluated carefully. If it is determined that peaks correspond to alleles and a potential genotype proceed to the Failure of Negative Controls section, below.

Failure of Negative Controls

Failure of negative controls can result from a variety of situations. Each failure will be evaluated on a case by case basis.

The point and source of the failure should be determined, when possible.

If the alleles and/or genotypes observed in the negative control(s) for a set of reactions are the same as the genotype that supports an inclusion, the data for those related samples is voided and may require re-extraction of the original item of evidence.

If the alleles or genotype observed are not associated with a genotype that supports an inclusion, the data may be interpreted cautiously. If the results are reported, the failed control must be reported in the written report.

Negative Control Failures

Performance failure in the Positive and/or Negative controls may require additional actions to be taken. Instances of performance failure shall be reported to the Unit Supervisor and DNA Technical Leader. Refer to the Quality Manual section for Discrepancies and Corrective Actions.

2.11.6 Step 6: REFERENCE DNA SAMPLE INTERPRETATION

Comparison of reference DNA profiles to evidence DNA profiles shall only be completed when the interpretation of the evidence DNA sample is complete. However, if the evidence sample is considered intimate the expected donor's profile may be used to assist with mixture deconvolution.

A single donor reference DNA sample, in most instances, will result in either homozygous (a single allele) or heterozygous (two alleles) results at each genetic marker tested. In contrast, in most instances the single Y-STR locus DYS391 will only show a single haplotype and the use of a Stochastic Threshold is generally not relevant. Single donor reference DNA samples must meet the Analytical Threshold of 250 RFUs. Homozygous loci, those that have a single allele, must meet the 900 RFUs Stochastic Threshold. In some rare instances, more than two alleles may be represented at a genetic marker of a single donor reference DNA sample. These are generally termed tri-allelic patterns. Tri-allelic markers are not used in statistical calculations, thus are not required to meet the 900 RFUs Stochastic Threshold. If a peak is detected that meets the criteria for pull-up in a reference DNA sample, but is labeled with an allele type (is within an allele bin), consideration must be given to the potential for peak imabalance or a tri-allelic pattern. A pull-up peak that is labeled as an allele may be interpreted as pull-up in a reference DNA sample if it can be reasonably determined that it did not result from a tri-allelic pattern or peak imbalance. It is acceptable to have heterozygous alleles below the 900 RFUs Stochastic Threshold as long as there are no indications of a mixture across the genetic profile. It is

acceptable, for single donor reference DNA samples, to have Peak Height Ratios (PHRs) less than the expected 50% for heterozygous markers greater than the 900 RFUs Stochastic Threshold. However, when Peak Height Ratios are less than the expected level(s), consideration should be given to the potential interpretation implications of the imbalance.

Reference Samples:

A results statement will be provided for each reference DNA sample analyzed prior to conclusion statements for evidentiary samples.

Criteria	Statement
Results at all markers tested	A DNA profile was obtained from ITEM 1
Results at <all markers tested, sufficient for comparison	A partial DNA profile was obtained from ITEM 1 that is sufficient for comparison purposes.
Results at <all markers tested, insufficient for comparison	A partial DNA profile was obtained from ITEM 1 that is not sufficient for comparison purposes.
No profile obtained	A DNA profile was not obtained from ITEM 1

2.11.7 Step 7: GENERAL CONSIDERATIONS FOR INTERPRETATION

Batch Comparisons

A comparison of DNA profiles within a batch of cases must be completed to determine the potential for contamination. Comparisons may be limited to DNA profiles/types that have probative value. Documentation of completion of this procedure must be included within the case file. Observations of potential contamination shall be reported to the Unit Supervisor and/or DNA Technical Leader.

STRMix Eligibility

All available loci and alleles ≥ the 250 RFU analytical threshold and below the maximum peak heights of 25,000 RFUs should be utilized during the initial interpretation. It should be noted that peaks not represented by an allele (artifacts) do not need to be included within the initial interpretation.

Data generated from the same extract and amplification may be interpreted across no more than two electropherograms for inclusion and exclusion determinations if the profiles obtained are consistent with one another. However, any given locus shall only be interpreted from a single electropherogram. STRMix™ has not been approved for use with multiple data sets from the same sample.

Based on a visual comparison of the DNA typing results, the DNA Analyst may conclude that a person of interest (POI) is excluded as a possible contributor if the observed alleles within the forensic sample DNA profile are not concordant with the POI DNA profile. These DNA profiles may not require STRMix™ software for further interpretations, but if the ability to exclude a person of interest is not free from ambiguity, STRMix™ ma y be used to assist with the inclusion/exclusion determination. STRMix™ may be utilized for CODIS suitability at the DNA Analyst discretion. For single source profiles, an exclusion is declared when one or more loci in a single-source evidentiary profile is different from that of a reference DNA profile. To declare an exclusion of a reference DNA profile from a mixed forensic sample DNA profile, the analyst should consider the number of contributors, the number and height of alleles detected per locus, the height of stutter peaks, the potential for allele sharing among contributors, and the potential for allele dropout. Generally, the greater the complexity of the typing results, the greater the potential that the result should be interpreted using STRMix™.

Based on visual comparison, the analyst may declare an exclusion to a mixed forensic sample DNA profile if the reference DNA profile is not consistent with any potential allele combinations in the mixture.

Criteria	Statement
1-2 alleles at all loci tested, exclusion	The DNA profile obtained from ITEM 1 is consistent with coming from a single individual. ITEM 2 is excluded as a possible donor to ITEM 1.
1-2 alleles at all loci tested, 1 additional minor allele at one locus, exclusion	The DNA profile obtained from ITEM 1 is consistent with coming from a single individual. ITEM 2 is excluded as a possible donor to ITEM 1. One additional DNA type foreign to ITEM 1 was obtained indicating an additional donor(s) or artifact.
1-2 alleles at all loci tested, 1 additional allele at one locus, possible tri-allele, exclusion	The DNA profile obtained from ITEM 1 is consistent with coming from a single individual. ITEM 2 is excluded as a possible donor to ITEM 1. A possible tri-allelic pattern was observed that was not considered in the interpretation.
1-2 alleles at <all loci tested, exclusion	The partial DNA profile obtained from ITEM 1 is consistent with coming from a single individual. ITEM 2 is excluded as a possible donor to ITEM 1.
1-2 alleles at <all loci tested, insufficient for comparison	A partial DNA profile was obtained from ITEM 1 that is insufficient for comparison purposes.

>2 alleles at many loci tested, exclusion *If STRMix™ analysis is used, you may add a row to the item table for Exclusions. If STRMix™ is not used, the statement may be used independently.*	ITEM 2 is excluded as a possible contributor to the mixed DNA profile obtained from ITEM 1.

STRMix™ analysis is not performed for exclusionary conclusions determined from visual comparisons unless deconvolution for CODIS entry is necessary. In addition, a match between a reference sample and a forensic sample DNA profile for which that individual's DNA can reasonably be expected to be present (intimate samples only-defined as internal body swabs, external body swabs and some clothing if there is documentation to support that the item was physically removed from the individual) does not require STRMix™ analysis unless the additional DNA types meet quality criteria for further STRMix™ analysis. Forensic sample DNA profiles may be imported into the STRMix™ software for further analysis. The overall completeness, quality and number of potential donors to any given forensic sample DNA profile must be considered prior to STRMix™ analysis. In general, forensic sample DNA profiles with 4 or fewer donors may be eligible for STRMix™ analysis. Forensic sample DNA profiles that exhibit excessive artifacts, very partial profiles with limited genetic data, more than 4 donors and/or elevated potential for allelic dropout at many/most genetic markers may not be suitable for STRMix™ analysis.

Criteria	Statement
DNA reference included on intimate sample	ITEM 2 was expected to be present within the DNA profile observed from ITEM 1. ITEM 1 is a mixture of DNA from more than one contributor including ITEM 2. The additional DNA types foreign to ITEM 2 are not sufficient for further interpretation and analysis; therefore, no comparison can be made to additional DNA reference samples.
≥5 contributors or significant artifacts or very partial profile or significant potential for dropout	The DNA profile obtained from ITEM 1 is not sufficient for further interpretation and analysis due to its complexity and number of potential contributors; therefore, no comparison can be made to DNA reference samples.

2.11.8 Step 8: STRMix™ Application

Generation of STRMix™ Input File

Open the GeneMapper® ID-X v1.4 software by logging into the software as DNA User. Open the project file previously generated using GeneMapper® ID-X v1.4. Select the appropriate analysis method (i.e. STRMix™ Casework or STRMix™ Reference), panel and size standard in the corresponding column and drop-down box. The analysis method incorporates the same thresholds and methods as used previously, except the stutter thresholds are removed for the STRMix Casework method. Select Analyze, or the green arrow.

The electropherograms may be reviewed a second time whereby labels may be removed from peaks resulting from artifacts such as fluorescent spikes, baseline elevation and/or elevated stutter (n+/n-). Upon review, print these "edited" electropherograms or utilize electronic capture methods.

STRMix™ requires use of a numerical value for the allele call. It will not accept "OL" or ">" as part of the allele call. If the DNA analyst is unable to accurately assign a numerical allele call to an OL allele due to migrational or binning concerns, the entire locus shall be removed from the STRMix™ table and analysis.

Tri-allelic loci cannot be included within the STRMix™ table and cannot be incorporated in the STRMix™ analysis.

Export the project into the designated location for backup by clicking Tools and then GeneMapper® Manager. Alternatively, depress the Control button and M at the same time (Ctrl+M). Select project from list and click Export. Select run folder, enter in project's name, and click Save. Click Done to exit out of GeneMapper® Manager. Electronic data shall be stored on an external electronic medium (i.e. CDs) at least quarterly and maintained. Upon review, print electropherograms or utilize electronic capture methods.

Once the electropherograms have been edited and no further changes are necessary, export a genotype table using the appropriate table setting (STRMix™ Casework or STRMix™ Reference) within GeneMapper® ID-X v1.4. Ensure that the number of alleles incorporated into the .txt file is sufficient to capture all of the alleles from each sample and is concordant with the STRMix™ software setting. 20 alleles is generally a sufficient number to capture all alleles for a 4 person mixture and should not be altered from this value.

Determining the Number of Contributors

The number of contributors to the DNA typing results is estimated after application of GMIDX filters and the removal of artifacts, if present. It is based on the number of alleles at each locus and the relative peak heights of alleles within a locus. An assumed number of contributors is required by STRMix™ in the interpretation of the typing results from evidentiary samples.

A sample is generally considered to have originated from a single individual if one or two alleles are present at all loci for which typing results were obtained and the PHR for all heterozygous loci are within empirically determined values. A sample that displays a heterozygous peak height imbalance at one or two loci, but for which no other results indicate the presence of a mixture, is generally considered a singe source sample. Samples in which three allelic peaks are observed at a single locus without any other indications of a mixture, may be concluded to be single

sourced. This conclusion should be based on the relative peak heights of the three peaks and the size range of the alleles that occur at that locus. A peak that exists in a minus stutter position that exceeds the expected stutter percentage may be interpreted as a stutter peak for purposes of determining the number of possible contributors. A peak in positive stutter may also be interpreted as a stutter peak in a single sourced sample if its height is <5% of the source peak. For STRMix™ analysis of single source samples, peaks in the negative stutter position remain in the STRMix™ input file while peaks that have been interpreted as positive stutter should be declared as artifacts and removed from the electropherogram.

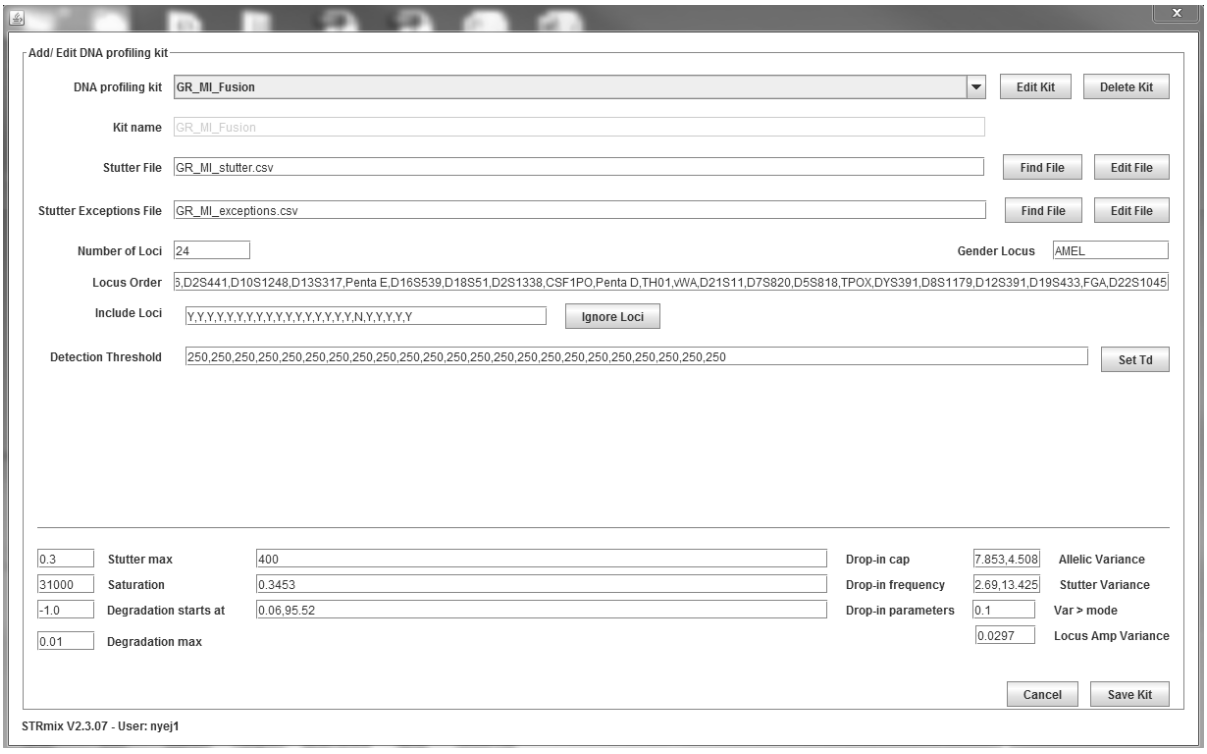
Mixtures are generally declared if three or more alleles are present at one or more loci and or the heterozygous peak height ratios are below expectations. The number of contributors to a mixed DNA sample shall be determined based upon the locus with the greatest number of interpretable alleles (≥ 250 RFUs) and documented within the case record. The number of contributors shall be determined by dividing the number of interpretable alleles at that locus by 2. The value shall be rounded up to the nearest whole number. It should be noted that this method only provides an estimate of the minimum number of contributors and the classification of any profile as a mixture and the number of contributors must be based on an evaluation of the profile in its entirety. The potential for degradation and allele drop-out should be considered when determining the number of possible contributors to a mixture. Additionally, the potential for allele sharing, pull-up and additive effects to stutter shall also be considered. Studies indicate it is difficult to determine with certainty the actual number of donors to any given mixture, especially as the number of donors increases. The true number of contributors to a mixed forensic sample is always unknown and can only be estimated for purposes of using STRMix^{TM}. For this reason, it may be necessary to analyze the sample using STRMix^{TM} under different contributor numbers. If STRMix^{TM} is used under different contributor estimates, each analysis shall be included within the laboratory report with the proper assumptions assigned to each analysis.

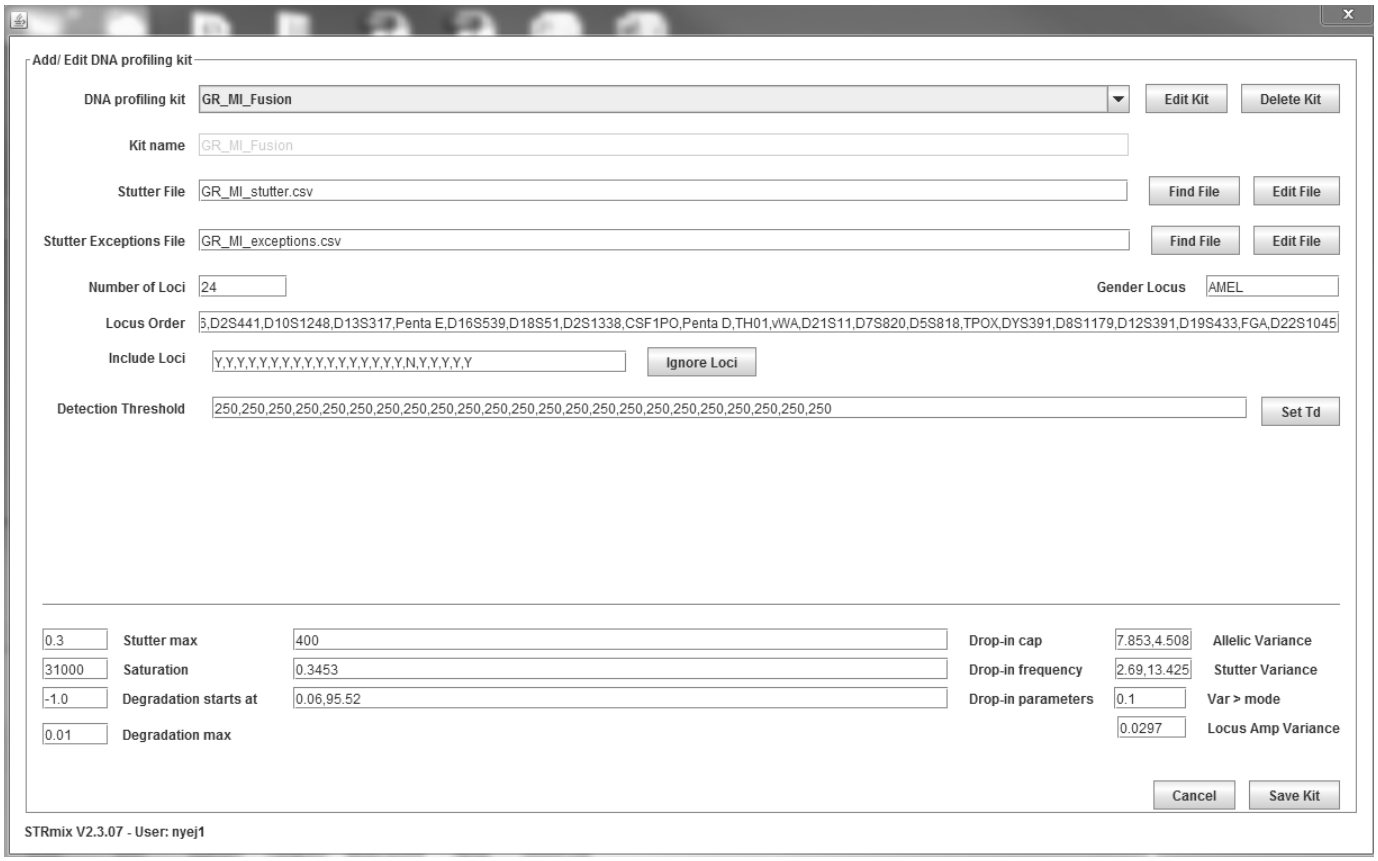
Criteria	Statement
Determined to be a single source sample	Interpretation of ITEM 1 was performed assuming that the DNA profile originated from a single individual.
Determined to be a mixed source sample	Interpretation of ITEM 1 was performed assuming that the DNA profile originated from XXX individuals.

STRMix Software

Version 2.3.07 of the STRMix™ software application is the accepted version for use in casework. All supporting formula may be found in the STRMix™ User's Guide.

STRMix™ software setting are represented below.





The value for the MCMC accepts is set to 100,000 for burn-in and 500,000 total and will not be changed without approval of the DNA Technical Leader. An F_{ST} setting of 0.01 (as used in the distribution equation) is used for the African American, Caucasian and Hispanic populations. Smaller populations, such as Native Americans shall use an F_{ST} value of 0.03. The population database may be found at:

Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) U.S. population data for 29 autosomal STR loci. *Forensic Sci. Int. Genet.* 7: e82-e83.

Propositions for Calculating the Likelihood Ratio

Two propositions must be established in STRMix™ prior to analysis. H_1 includes the POI, and may include known individuals on intimate samples, consenting sexual partner(s) or unknown individuals. For example, for a sexual assault with a single assailant the H_1 proposition may consist of the POI and the victim. Or for a three person mixture, the H_1 proposition may consist of the POI and two unrelated unknown individuals. The second proposition is commonly referred to as H_2, and consists of the number of unrelated individuals equaling the total number of contributors to the sample and may include the known contributor from an intimate sample or consenting sexual partner(s). For example, for a sexual assault with a single assailant the H_2 proposition may consist of the victim and one unknown unrelated contributor or for a three person mixture the H_2 proposition may consist of three unrelated unknown individuals.

For cases with multiple POIs, each POI reference profile is analyzed individually in STRMix and reported separately. A request or scenario where it may be informative to report the LR of combined POIs must be pre-approved by the DNA Technical Leader. For example, a two person mixture with two POI reference profiles shall be evaluated and reported as an H_1 of POI_1 plus an Unknown and an H_2 of two Unknowns and an H_1 of POI $_2$ plus an unknown and an H_2 of two unknowns, individually. DNA Technical Leader approval is required to use an H_1 of POI_1 and POI_2 and an H $_2$ of two Unknowns.

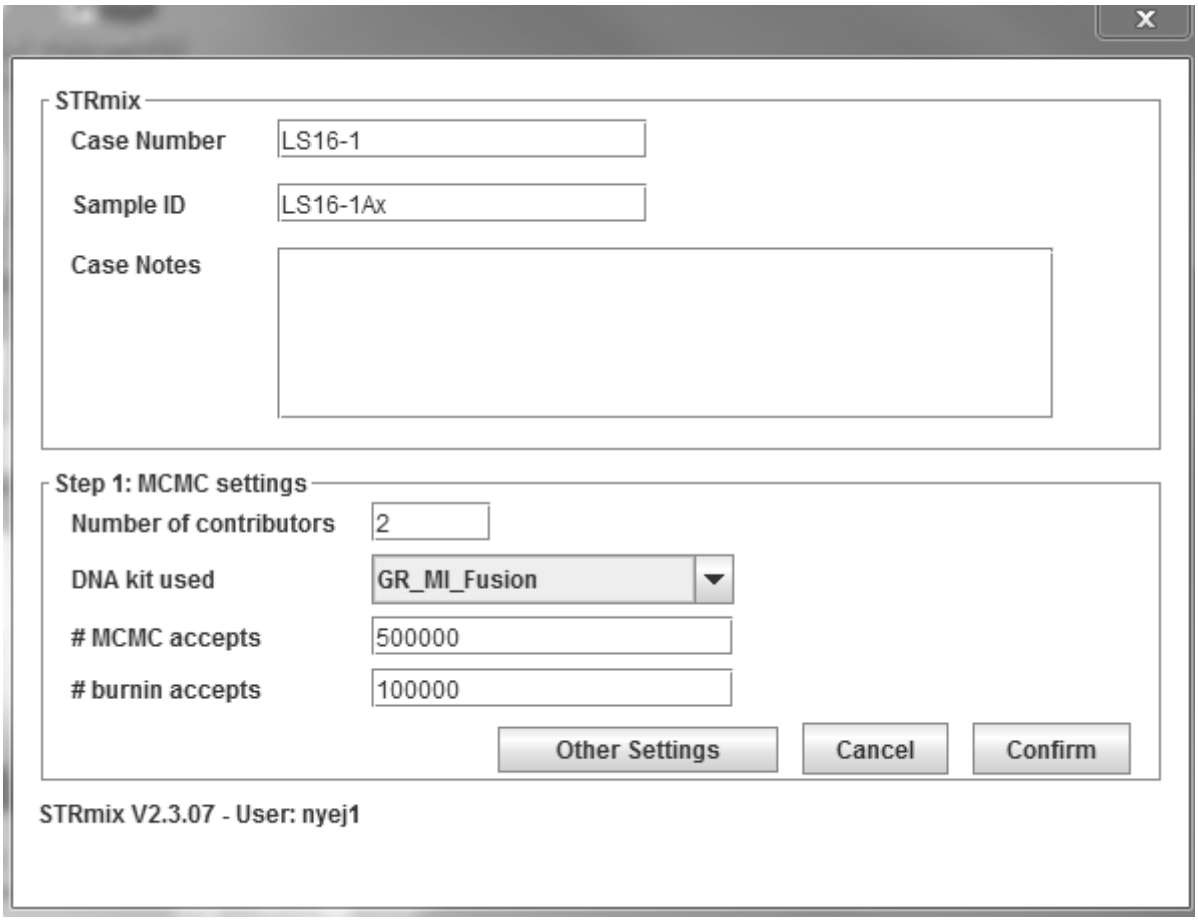
Assuming a contributor to a sample shall be limited to intimate samples or consenting sexual partners with proper case file documentation.

STRMix™ Analysis

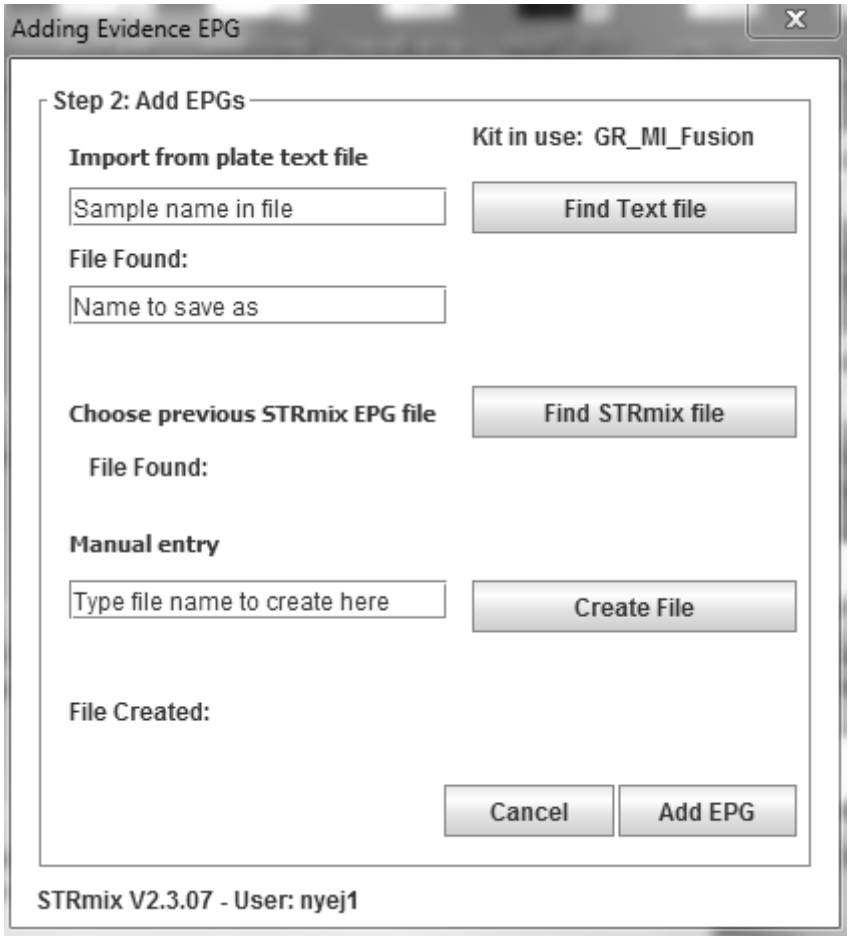
The STRMix™ application is launched by double clicking the shortcut. Once the application is running, The START MIXTURE ANALYSIS icon is

clicked.

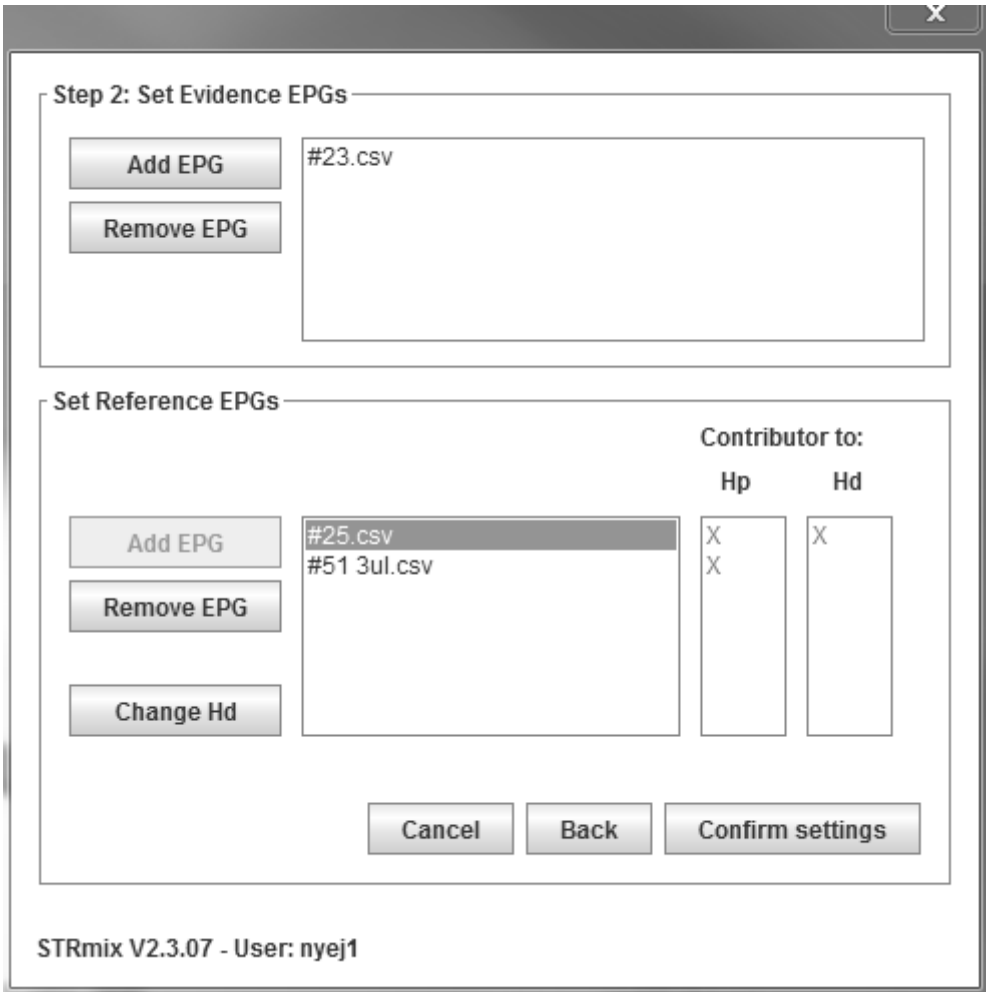
A new screen will appear whereby the case and sample specific information must be filled out.



The number of contributors must be entered. Select CONFIRM. Another screen opens where the electropherogram (EPG) of the forensic DNA profile is uploaded to the software by selecting ADD EPG.



By selecting ADD EPG, another screen opens. Select FIND TEXT FILE and browse to the .txt file exported from GMIDx that contains the forensic DNA profile you wish to analyze in STRMix™.

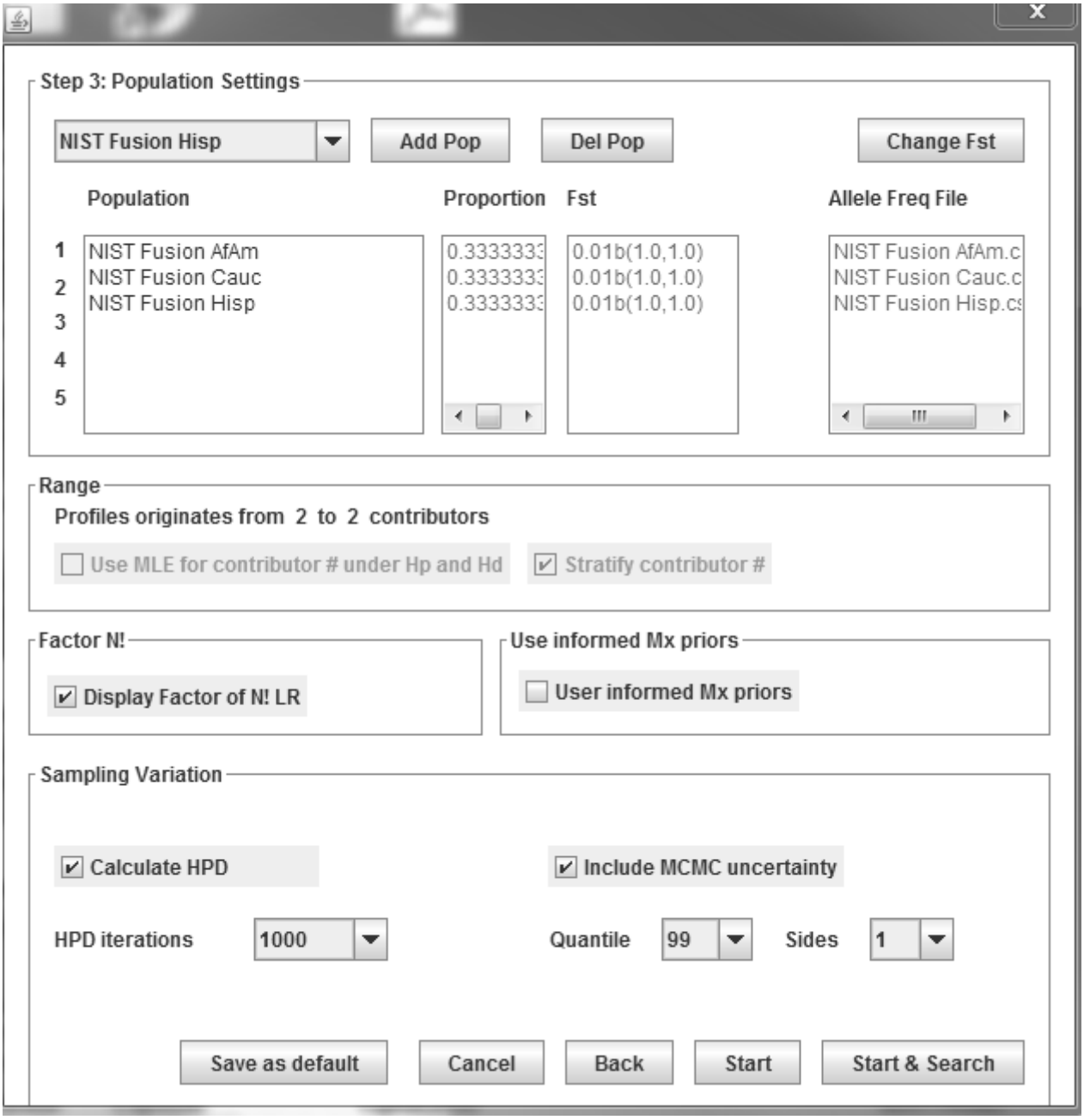


Similarly, reference DNA profiles from intimate samples (assumed) and/or POIs may be uploaded in the section entitled Set Reference EPGs. The CHANGE H_d icon may be selected when a reference sample is highlighted to change the H_1 and H_2 hypotheses.

Evidentiary specimens imported into STRMix™ should include all allelic peaks, including all peaks in the negative stutter position. Tri-allelic loci should be excluded from STRMix™ analysis during the IDx interpretation. Forward stutter and forward/reverse additive stutter is not modeled in STRMix™ version 2.3.07. For that reason, if a peak on the electropherogram is interpreted as arising from an artifact after considering the number of potential donors and the overall DNA profile, it may be removed from STRMix™ consideration during the IDx interpretation. If an allele is removed, the following statement shall be included in the laboratory report.

Criteria	Statement
Allele removed from STRMix™ table	An additional allele(s) was observed that may be an artifact or additional contributor. It was not included in the interpretation.

Once the case and sample information are filled out and the profile(s) is added to STRMix™ with the necessary propositions, select confirm and a screen will open for selecting the population databases. Generally, the African American, Caucasian and Hispanic populations should be selected.



Select START and allow the analysis to complete. Once the analysis is complete, select RUN REPORT to create an advanced report.

Additionally, STRMix™ analysis can be completed in a batch mode using the BATCH MODE icon. Profiles can be added and deleted to the "batch" prior to initiating the batch analysis. Similarly, a previously analyzed profile by STRMix™ can be compared to a subsequent reference sample for generation of a likelihood ratio through selection of the LR FROM PREVIOUS ANALYSIS icon. The .ini file from the initial deconvolution must be selected and likelihood ratio propositions set up as previously described.

The DNA analyst will evaluate the STRMix™ results by examining the weights of the various genotypes and individual locus LRs relative to the DNA profile being analyzed in STRMix™. In general, a STRMix™ analysis should not be repeated, except for alternate hypotheses or when a STRMix™ analysis has produced a result that requires further investigation. Examples requiring an additional STRMix™ analysis may include:

- An LR=0 for a single locus or very few loci when other loci have an LR>0, when the reference DNA profile is consistent with the evidentiary profile. After reviewing the genotype probabilities, it may indicate that STRMix™ did not consider all of the potential genotypes at that given locus. Repeat analysis should be completed with the same STRMix™ settings.
- An LR=0 for a single locus or very few loci when other loci have an LR>0, when the reference DNA profile is consistent with the evidentiary profile. Review of the electropherogram indicates that an allele was not included in the STRMix™ input file. In this instance, the analysis should be repeated with the same STRMix™ settings with the locus ignored in STRMix™.
- An observation that does not appear intuitive, such as a mixture proportion that does not reflect what is observed in the typing results, degradation that does not reflect what is observed and/or the interpreted contributor genotypes do not appear intuitively correct.
- The number of contributors to a sample is ambiguous, or the STRMix™ results are not intuitively correct under the assumption of the tested number of contributors. The DNA analyst may repeat the STRMix™ analysis assuming a different number of contributors.

If STRMix™ analysis has been repeated, all analyses will be included in the case file and report unless approval is provided by the Unit Supervisor and/or DNA Technical Leader. The files shall include the Advanced Report and all STRMix™ analysis files saved to the corresponding run folder for all analyses generated by STRMix™. If a STRMix™ analysis is not included in the report, upon receiving approval, a notation in the laboratory report shall indicate additional analyses were completed that did not meet reporting standards as noted below.

The single lowest LR obtained across all populations used in the calculation will be reported.

Additional diagnostics information within the STRMix™ output **may** provide additional insight regarding the effectiveness of the evaluation, but a

review of the locus LR values and genotypic weights shall remain the main diagnostic evaluation of STRMix™.

- The value displayed for total iterations indicates the total number of post burn-in iterations that the MCMC ran during its analysis. This value, along with the number of accepts can be informative. This value does not provide a diagnostic that the MCMC has converged, it can inform the user on the clustering of the parameter values about a specific point in order to be accepted. A low acceptance rate (e.g. 1 in thousands to millions) on its own is not an indication that rework is required.
- The average log likelihood is the average probability value created at each of the post burn-in MCMC iterations. The larger this value the better STRMix™ was able to describe the observed data. A negative or very low value suggests STRMix™ was not able to describe the data very well and requires additional scrutiny. Low average log likelihood values alone are not necessarily an indicator of an issue.
- The Gelman-Rubin convergence value describes if the independent MCMC chains have converged and spent time in the same space. Version 2.3.07 of STRMix™ does not rely as heavily on the Gelman-Rubin convergence value as previous versions of the software, but a value of 1.2 or less generally describes MCMC chains that have converged. Values greater than 1.2 describe an STRMix™ analysis that may not have converged and may require additional scrutiny.
- Allele and stutter variance constants can be used as a guide as to the level of stochastic variation in peak heights that is present in the profile. Used in conjunction with the average log likelihood, a large variance or stutter variance constant can indicate poor PCR. A low average log likelihood and an average variance constant can simply mean the sample is low level.

The following conclusion may be used if the DNA analyst evaluation indicates an unacceptable STRMix™ analysis:

Criteria	Statement
Incomplete STRMix™ analysis	The results of ITEM 1 do not meet quality criteria for use in statistical calculations; therefore, no comparison can be made to DNA reference samples.
Unacceptable STRMix™ an alysis	Additional analyses of Item 1 were performed that did not meet quality control criteria; therefore, they were not reported.

CODIS

Forensic unknown profiles may be entered into CODIS directly. Forensic mixtures may be deconvoluted using STRMix™ to determine the CODIS profile. Any contributor generated from a STRMix™ analysis may be entered into CODIS if at each given locus, the genotype(s) exhibit a combined weight of 0.99 or greater and the CODIS criteria for Match Estimator is met. For example

Contributor 1

Genotype	Weight
8,8	0.23
8,10	0.57
10,10	0.15
7,10	0.04

The total weight of the four genotypes is 0.99. Therefore, a 7,8,10 could be entered into CODIS for this locus.

Reporting STRMix™ Results and Conclusions

The following statement shall be included in all STR reports to indicate which loci were utilized:

> *DNA recovered from the above submitted samples was processed using the polymerase chain reaction (PCR) and the PowerPlex® Fusion system.*

As applicable, the following statement shall be included:

> *These profiles were evaluated using STRMix™, a probabilistic genotyping software application.*

For any given STRMix™ analysis, the assumption as to the number of contributors and any individuals assumed to be present in the sample shall be reported.

The TOTAL LR value from the STRMix™ report will be used.

For a TOTAL LR value of 0, an exclusion is reported in the laboratory report.

For a set of LR values greater than 1 for a given STRMix™ analysis, the single lowest LR across all populations for reporting.

For a set of LR values greater than 0 and less than 1, the H_2 hypothesis has more support than the H_1 hypothesis. For this reason, the reciprocal of the single lowest LR across all populations is calculated. The reciprocal LR shall be reported to indicate it favors H_2. The corresponding Qualitative Equivalent as it relates to the reciprocal LR value shall be incorporated into the conclusions. As an example, a TOTAL LR value of 0.0008 provides a reciprocal LR value of 1250 and a Qualitative Equivalent of *Strong Support to the H_2 hypothesis*.

All LR values that are reported should include two significant digits in the direction that is the most conservative value. For example:

- 188 Trillion would be reported as 180 Trillion
- 27.2 Quadrillion would be reported as 27 Quadrillion
- 184,987 would be reported as 180,000

The following statement shall be included in all STR reports that include a STRMix analysis:

The magnitude of the likelihood ratio relates to the degree of support provided by the evidence under the tested hypotheses and assumptions. The qualitative statement for a comparison of the evidentiary typing results to a person of interest is based on the following table.

LR	Qualitative Equivalent
0	Exclusion
>0 to 99	Uninformative
100 to 999	Moderate Support
1,000 to 9,999	Strong Support
10,000 greater	Very Strong Support

Criteria	Statement
Exclusion without a conditioned contributor(s)	ITEM 2 is excluded as a possible contributor to the DNA obtained from ITEM 1.
Exclusion with a conditioned contributor(s)	ITEM 2 was assumed to be a contributor to ITEM 1. ITEM 3 is excluded as a possible contributor to the DNA obtained from ITEM 1.

Reporting an LR that supports H_2 is reported as follows:

Criteria	Statement
Support of H_2 wit hout a conditioned contributor(s)	Based on the DNA typing results obtained, it is at least XXX times more likely if the DNA observed in ITEM 1 originated from XXX unrelated unknown individuals than if the DNA originated from ITEM 2 and XXX unrelated unknown individuals. This analysis provides XXX support that ITEM 2 is not a contributor to the DNA typing results obtained from ITEM 1.
Support of H_2 wit h a conditioned contributor(s)	ITEM 2 was assumed to be a contributor to ITEM 1. Based on the DNA typing results obtained, it is at least XXX times more likely if the DNA originated from ITEM 2 and XXX unrelated unknown individual(s) than if they originated from ITEM 2 and ITEM 3. This analysis provides XXX support that ITEM 2 is not a contributor to the DNA typing results obtained from ITEM 1.

Reporting an LR = 1, indicating H_1 and H_2 are the same is reported as follows:

Criteria	Statement
Equal support to H_1 and H_2 without conditioned conributor(s)	Based on the DNA typing results obtained, it is equally likely if it originated from ITEM 1 and XXX unrelated unknown individuals or if it originated from XXX unrelated individuals. Therefore, the analysis of ITEM 1 with regards to ITEM 2 is uninformative.
Equal support to H_1 and H_2 with conditioned conributor(s)	ITEM 2 was assumed to be a contributor to ITEM 1. Based on the DNA typing results obtained, it is equally likely if it originated from ITEM 2 and ITEM 3 or if it originated from ITEM 2 and an unrelated unknown individual. Therefore, the analysis of ITEM 1 with regards to ITEM 2 is uninformative.

Reporting an LR greater than 1

Criteria	Statement
Without a conditioned contributor(s)	Based on the DNA typing results obtained for ITEM 1, it is at least XXX times more likely if it originated from ITEM 2 and an unrelated unknown individual(s) than if it originated from XXX unrelated unknown individual. This analysis provides XXX support that ITEM 2 is a contributor to the DNA typing results obtained from ITEM 1.

With a conditioned contributor(s)	ITEM 2 was assumed to be a contributor to ITEM 1. Based on the DNA typing results obtained from ITEM 1, it is at least XXX times more likely if it originated from ITEM 2 and ITEM 3 than if it originated from ITEM 2 and an unrelated unknown individual. This analysis provides XXX support that ITEM 3 is a contributor to the DNA typing results obtained from ITEM 1.

Remarks Statements:

Criteria	Statement
CODIS entry	A DNA profile from ITEM 1 was entered into the COmbined DNA Index System (CODIS).
CODIS entry no, sample	The DNA profile from ITEM 1 was not entered into the COmbined DNA Index System (CODIS).
CODIS entry no, case	DNA profiles were not entered into the COmbined DNA Index System (CODIS).
Reference sample request	Please submit a known buccal swab collected from potential donors to this case to be used for further analysis and comparisons.
All STRMix^{TM} analyses shall include this remark	The propositions were formed from the information available to the undersigned at the time of analysis. If this information changes or if other propositions should be considered, the analyst is able to undertake them if instructed with sufficient time.
Other staff members involved in FA Case Record	Other members of the Forensic Science Division may have processed evidence associated with this report, in addition to the reporting analyst.

2.12 DNA Extracts and Amplified Product

2.12 DNA Extracts and Amplified Product

2.12.1

Dilutions prepared from concentrated DNA extracts, where there is sufficient remaining stock extracted DNA to return to the investigating agency, and extracts created during the direct amplification preparation shall be considered work product and may be discarded upon completion of the technical and administrative reviews. The return of stock DNA extracts shall be handled in accordance with Biology Procedure manual section 2.15.

2.12.2

Extracted DNA shall be maintained in the following manner to minimize degradation.

2.12.2.1

Pending further analysis, DNA extracts shall be stored for short term (overnight)at 4°C.

2.12.2.2

Samples to be stored for longer than overnight shall be placed into a freezer at -20°C.

2.12.3

Amplified product shall be maintained in the following manner to minimize degradation:

2.12.3.1

Amplified product shall be stored in a freezer at -20°C.

2.12.3.2

Amplified product may degrade over time and can be discarded upon completion of technical and administrative reviews.

2.12.4

Stock (original) DNA extracts are considered evidence. DNA dilutions prepared from stock DNA extracts are considered work product. Amplified product shall be considered work product.

2.14 DNA Report

2.14 DNA Documentation-Laboratory Report and Case Materials

2.14.1

Laboratory reports shall be generated according to the Forensic Science Division Quality Manual.

2.14.2

The report header and body shall be built utilizing the Laboratory Information Management System.

2.14.3

The body of the report shall include all evidence received, the genetic loci tested, conclusions supported by the generated data, disposition of evidence and statistical estimations (as needed).

2.14.4

All reports will be peer and administratively reviewed with the following exception:

2.14.4.1

Cases where an analysis was not conducted may only require an administrative review (i.e. agency withdrew laboratory examination request).

2.14.4.2

The report release date shall be considered the "Date of Issue."

2.14.5

Abbreviations used within the case notes and worksheets, that are not present in Appendix 1, shall be accompanied by an abbreviations key in each case jacket.

2.14.6

Laboratory worksheets for each portion of the testing shall be maintained in the case file including the .aan file produced during quantification of DNA. This may include the following worksheets: Evidence Itemization, DNA Extraction, DNA Quantitation, Amplification and STR DNA Mixture Interpretation. Additionally, the electropherograms used for the interpretation and for the positive and negative controls shall be maintained in the case file. A more detailed description can be reviewed in DNA Procedure 2.6.